Genet. Res., Camb. (1981), **38**, *pp.* 251–266
With 7 text-figures
Printed in Great Britain

Chromosomal locations of *catA*, *pobA*, *pcaA*, *dcu* and *chu* genes in *Pseudomonas aeruginosa*

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(*Received* 5 *January* 1981 *and in revised form* 6 *May* 1981)

SUMMARY

Eleven catabolic markers have been located on the chromosome of *Pseudomonas aeruginosa* PAO using FP5-mediated conjugation and G101 transduction. Most of these markers are located in the region 20–35 min, and the remainder in the region later than 60 min. Four *chu* genes concerned in the sequential degradation of choline to glycine are closely linked.

1. INTRODUCTION

Pseudomonas aeruginosa is known to utilize a wide variety of substrates for growth (Clarke & Richmond, 1975). Genetic studies, mainly with biosynthetic markers, have located about one hundred gene loci on the chromosome of *P. aeruginosa* PAO (Royle *et al.* 1981). In this paper, the chromosomal location of eleven genes participating in the utilization of benzoate, *p*-hydroxybenzoate, dicarboxylic acids and choline is described. The approximate position of one of these genes, *catA*, has been presented previously (Matsumoto *et al.* 1978).

2. MATERIALS AND METHODS

(i) *Bacterial strains*

The strains used are listed in Table 1. They are all derivatives of *P. aeruginosa* strain PAO (Holloway, 1969). Three new gene symbols have been used; *chu*, choline utilization (*chuA*, choline dehydrogenase, *chuD*, dimethylglycine dehydrogenase, *chuE*, sarcosine dehydrogenase); *dcu*, dicarboxylic acid utilization, and *pyu*, pyrimidine utilization. These designations follow the nomenclature proposed by Holloway *et al.* (1979).

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Table 1. P. aeruginosa *strains used in this study*

Strain	Genotype*	Derivation	Reference or source
PAO1	Prototroph	Wild-type isolate	Holloway (1969)
PAO1816	*his-9004*, FP5$^+$	*his* and FP5$^+$ derivative of PAO1	Matsumoto & Tazaki (1975)
PAO1834	*met-9020*	*met* derivative of PAO1	This paper
PAO2146	*his-9004*, *leu-9008*, FP5$^+$	*leu* derivative of PAO1816	Matsumoto *et al.* (1978)
PAO2152	*his-9004*, *nir-9006*, FP5$^+$	*nir* derivative of PAO1816	Matsumoto *et al.* (1978)
PAO2175	*met-9020*, *catA1*	*cat* derivative of PAO1834	Matsumoto *et al.* (1978)
PAO2178	*met-9020*, *catA1*, *nar-9011*	*nar* derivative of PAO2175	Matsumoto collection
PAO2192	*met-9020*, *catA1*, *nar-9011*, *trp-9031*, *chuE9002*	*chuE* derivative of PAO2364	This paper
PAO2194	*met-9020*, *catA1*, *nar-9011*, *trp-9031*, *chuE9002*, *lys-9016*	*lys* derivative of PAO2192	This paper
PAO2302	*met-9020*, *catA1*, *puuA1*	*puuA* derivative of PAO2175	Matsumoto *et al.* (1978)
PAO2305	*met-9020*, *catA1*, *puuA1*, *trp-9026*	*trp* derivative of PAO2302	Matsumoto collection
PAO2362	*met-9020*, *catA1*, *puuA1*, *trp-9026*, *pobA9051*	*pobA* derivative of PAO2305	This paper
PAO2364	*met-9020*, *catA1*, *nar-9011*, *trp-9031*	*trp* derivative of PAO2175	Matsumoto collection
PAO2372	*met-9020*, *catA1*, *puuA1*, *trp-9026*, *pcaA9016*	*pcaA* derivative of PAO2305	This paper
PAO2374	*met-9020*, *catA1*, *puuA1*, *trp-9026*, *pobA9051*, *ilv-9018*	*ilv* derivative of PAO2362	This paper
PAO2375	*met-9020*, *catA1*, *nar-9011*, *mtu-9002*	*mtu* derivative of PAO2178	Royle *et al.* (1981)
PAO2376	*met-9020*, *catA1*, *nar-9011*, *mtu-9002*, *tyu-9030*	*tyu* derivative of PAO2375	Royle *et al.* (1981)
PAO2389	*met-9020*, *catA1*, *nar-9011*, *trp-9031*, *chuA9003*	*chuA* derivative of PAO2364	This paper
PAO2390	*met-9020*, *catA1*, *nar-9011*, *trp-9031*, *chuA9003*, *chu-9051*	*chu* derivative of PAO2389, with double mutations for *chu*	This paper
PAO2391	*met-9020*, *catA1*, *nar-9011*, *trp-9031*, *chuA9003*, *chuD9052*	*chuD* derivative of PAO2389, with double mutations for *chu*	This paper

Table 1. *continued*

PAO2392	*met-9020*, *catA1*, *nar-9011*, *trp-9031*, *chuA9003*, *chuE9053*	*chuE* derivative of PAO2389, with double mutations for *chu*	This paper
PAO2397	*met-9020*, *catA1*, *nar-9011*, *trp-9031*, *chuD9008*	*chuD* derivative of PAO2364	This paper
PAO4010	*met-9020*, *catA1*, *nar-9011*, *trp-9031*, *chu-9012*	*chu* derivate of PAO2364	This paper
PAO4016	*met-9020*, *catA1*, *nar-9011*, *trp-9031*, *chu-9012*, *chuD9054*	*chuD* derivative of PAO4010, with double mutations for *chu*	This paper
PAO4017	*met-9020*, *catA1*, *nar-9011*, *trp-9031*, *chu-9012*, *chuE9055*	*chuE* derivative of PAO4010, with double mutations for *chu*	This paper
PAO4020	*his-9004*, *nir-9006*, *str-9002*, FP5$^+$	Spontaneous streptomycin resistant derivative of PAO2152	This paper
PAO4032	*met-9020*, *catA1*, *nar-9011*, *mtu-9002*, *tyu-9030*, *dcu-9013*	*dcu* derivative of PAO2376	This paper
PAO4044	*met-9020*, *catA1*, *nar-9011*, *mtu-9002*, *tyu-9030*, *dcu-9041*	*dcu* derivative of PAO2376	This paper
PAO4101	*pyu-9010*	*pyu* derivative of PAO1	Matsumoto collection
PAO4102	*pyu-9010*, *trp-9051*	*trp* derivative of PAO4101	Matsumoto collection
PAO4104	*pyu-9010*, *trp-9051*, *dcu-9008*	*dcu* derivative of PAO4102	Matsumoto collection
PAO4105	*pyu-9010*, *trp-9051*, *dcu-9008*, *ilv-9048*	*ilv* derivative of PAO4104	This paper

* *Anabolic marker*: *his*, histidine; *leu*, leucine; *lys*, lysine; *met*, methionine; *trp*, tryptophan; *ilv*, isoleucine + valine.

Catabolic marker: *catA*, catechol 1,2-oxygenase; *chuA*, choline dehydrogenase; *chu-9012*, a biochemically unidentified defect in choline utilization; *chuD*, dimethylglycine dehydrogenase; *chuE*, sarcosine dehydrogenase; *dcu*, dicarboxylic acid utilization; *mtu*, mannitol utilization; *nar*, nitrate (to nitrite) reduction; *nir*, nitrite reduction; *pcaA*, protocatechuate 3,4-oxygenase; *pobA*, *p*-hydroxybenzoate hydroxylase; *puuA*, adenine deaminase; *pyu*, pyrimidine utilization; *tyu*, tyrosine utilization.

Other markers: *str*, streptomycin resistance.

Independent isolates of certain markers have been shown to be closely linked (allelic) to previously mapped markers (data not shown). In the following list, the previously mapped marker is given first; *hisI* is closely linked to *his-9004* (Matsumoto *et al.* 1978); *leu38* is closely linked to *leu-9008* and *leu-9018*; *met-9011* is closely linked to *met-9020*; *ilvD* is closely linked to *ilv-9018* and *ilv-9048*; *trp-9051* is closely linked to *trpE*, *trp-9051* responds equally well to anthranilate, indole and tryptophan; *trp-A,B* is closely linked to *trp-9026*, *trp-9029* and *trp-9031*, the latter three *trp* mutants respond to tryptophan alone, and accumulate indole; *tyu9009* is closely linked to *tyu-9030*; *nar-9011* and *nir-9006* may correspond to *narA,D* and *nirB*, respectively (Matsumoto *et al.* 1978).

(ii) *Media*

The complete medium used was nutrient broth or nutrient agar. The minimal medium was that of Ornston & Stanier (1966) but without nitrilotriacetic acid. Where appropriate, the test growth substrate was added at a concentration of 0·1 % (w/v) in place of glucose and/or ammonium sulphate. The minimal medium was supplemented, when required, with amino acid(s) at a concentration of 10^{-4} M.

(iii) *Isolation of catabolic mutants*

The cells in exponential phase of growth were treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (30 μg/ml for 15 min at 37 °C in acetate buffer, pH 5·8), incubated at 37 °C for 3 h in nutrient broth to allow expression of the mutations, and then starved in saline at 37 °C for 8 h. The starved cells were incubated at 37 °C in minimal medium containing the appropriate substrate and carbenicillin (1000 μg/ml). In the case of dicarboxylic acids, incubation was held at 30 °C because the substrates were utilized more rapidly than at 37 °C. The time of incubation varied with the substrate used. After the carbenicillin-contraselection, aliquots of the samples were plated onto nutrient agar, grown overnight, and the colonies which developed were replica-plated onto two minimal plates differing in their carbon and/or nitrogen sources; one contained the test substrate to be utilized by the mutants sought, and the other contained a compound which would support growth. Any colony that grew on the latter medium but was absent from the test substrate plate was subcultured and further characterized. The basis for the initial identification of each kind of mutant was as follows; the *catA* mutant was isolated as being unable to utilize benzoate as sole source of carbon, while the *pobA* and *pcaA* mutants were unable to grow on *p*-hydroxybenzoate and/or protocatechuate, respectively. Selection of *dcu* mutants was performed by screening for mutants that could utilize benzoate and *p*-hydroxybenzoate but not pimelate and/or adipate. The *chu* mutants were found among the clones that did not respond to choline, betaine, dimethylglycine and/or sarcosine. Mutants with double *chu* mutations were isolated by serial mutagenesis and selection using an appropriate substrate of degradative intermediates of choline. A *pyu* mutant was isolated by scoring colonies that could not grow on uracil but did grow on β-alanine, both compounds being added as sole sources of nitrogen. Auxotrophic mutants were isolated as previously described (Glover, 1968).

(iv) *Biochemical characterization of mutants*

(1) *Growth response test.* A saline suspension of cells (1×10^8 cell/ml) from a fresh nutrient agar culture was spread onto minimal plates containing the substrate to be tested. The results were read after incubation at 32 °C or at 37 °C for 2 days.

(2) *Enzyme assays.* Assays of catechol 1,2-oxygenase (*catA*), *p*-hydroxybenzoate hydroxylase (*pobA*) and protocatechuate 3,4-oxygenase (*pcaA*) activities were carried out as described by Gibson (1971). Cells were grown to the mid-exponential phase in nutrient broth containing 0·1 % (w/v) of a suitable aromatic compound

as an inducer. These cells were then disrupted in a sonicator, and a cell free extract was prepared by centrifuging at 12000 × *g* for 20 min at 2 °C. One unit of enzyme activity was defined as that amount of enzyme that either oxidized 1 μmole of catechol (*catA*), 1 μmole of NADPH (*pobA*), or 1 μmole of protocatechuate (*pcaA*), per min at 25 °C. Activities of choline dehydrogenase (*chuA*), dimethylglycine dehydrogenase (*chuD*) and sarcosine dehydrogenase (*chuE*) were assayed by following the reduction of dichlorophenolindophenol (DCIP) as described by Bater & Venables (1977) using membrane preparations from cells grown aerobically to the mid-exponential phase in nutrient broth with 0·2% (w/v) of choline, dimethylglycine or sarcosine as inducer. One unit of these enzyme activities was defined as that amount of enzyme that reduced 1 μmole of DCIP per min at 25 °C. Specific activities of enzymes are expressed as units per mg of protein. Protein concentration was estimated by the method of Lowry *et al.* (1951).

(v) *Genetic methods*

Conjugation was initiated by mixing broth cultures of donor and recipient in exponential phase (5×10^8/ml) at ratios ranging from 1:2 to 1:10, the mixtures being incubated at 37 °C for 2–3 h without agitation. The mixtures were then centrifuged, resuspended in saline to the original volume, and aliquots spread on selective plates. For transduction, a G101 phage suspension (ca. 10^{10} plaque forming units/ml) was mixed with an equal volume of broth culture of the recipient (1×10^9 cell/ml) in late exponential phase, and incubated at 37 °C for 15 min. The mixture was then centrifuged, the cells resuspended in an appropriate volume of saline, and aliquots plated onto selective plates. Recombinant colonies, obtained after incubation at 37 °C for 2–4 days, were purified on nutrient agar plates by single colony isolation and then tested for their inheritance of selected and unselected marker(s). Reduction of nitrate and nitrite was tested as described previously (Matsumoto *et al.* 1978). Selection of recombinants for catabolic markers was performed on minimal plates containing the appropriate substrate as either the sole source of carbon and/or nitrogen. Plates containing benzoate were used for the selection of $catA^+$ recombinants, and plates with *p*-hydroxybenzoate were used for both $pobA^+$ and $pcaA^+$ selection. Selection for dcu^+ was made on plates containing pimelate, and chu^+ recombinants were obtained on plates containing either choline, betaine, dimethylglycine or sarcosine. Isolation of pyu^+ was done on plates containing uracil.

3. RESULTS

(i) catA, pobA *and* pcaA *mutants*

Catechol, formed from benzoate, is converted to *cis, cis*-muconate by the action of catechol 1,2-oxygenase (*catA*), while *p*-hydroxybenzoate is degraded to protocatechuate by *p*-hydroxybenzoate hydroxylase (*pobA*) and then to γ-carboxymuconate by protocatechuate 3,4-oxygenase (*pcaA*). A preliminary mapping of these genes by means of transduction was carried out by Kemp &

Table 2. *Growth responses and Enzyme activities of* catA, pobA *and* pcaA *mutants*

Strain	Relevant mutation	Utilization of substrate*						Specific activity (units/mg)		
		Glu	Ant	Mdl	Ben	Pob	Pca	Catechol 1,2-oxygenase (Ben)†	*p*-hydroxybenzoate hydroxylase (Pob)	Protocatechuate 3,4-oxygenase (Pca)
PAO1834	*catA*$^+$, *pobA*$^+$, *pcaA*$^+$	+	+	+	+	+	+	0·034	0·0045	0·0092
PAO2152	*catA*$^+$, *pobA*$^+$, *pcaA*$^+$	+	+	+	+	+	+	0·039	0·0041	0·0109
PAO2175	*catA1*	+	−	−	−	+	+	0·001	0·0037	0·0101
PAO2374	*catA1*, *pobA9051*	+	−	−	−	−	+	nd	< 0·0001	0·0095
PAO2372	*catA1*, *pcaA9016*	+	−	−	−	−	−	nd	0·0032	< 0·0001

+, growth occurred; −, no growth; nd, not determined.

* Abbreviation: Glu, glucose; Ant, anthranilate; Mdl, mandelate; Ben, benzoate; Pob, *p*-hydroxybenzoate; Pca, protocatechuate.

† Substrate shown in parentheses was added as an inducer for each enzyme.

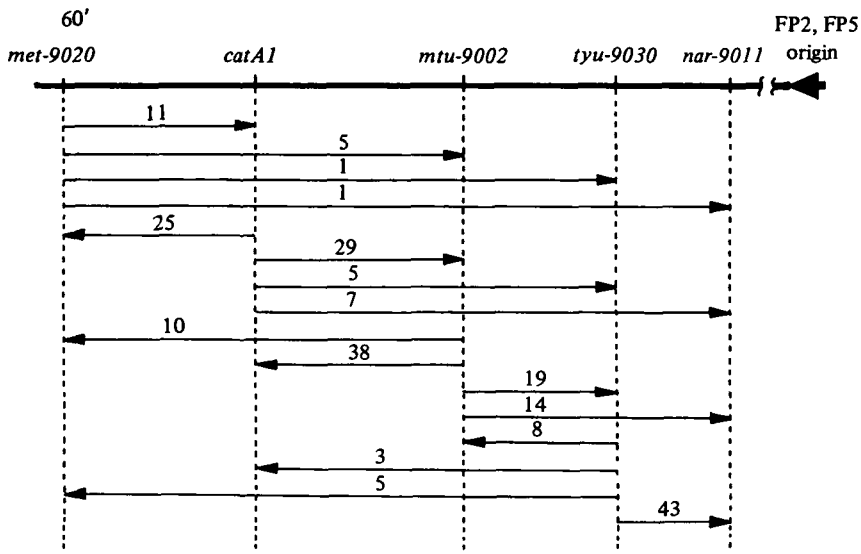

Fig. 1. Linkage values obtained from the mating (PAO2146, FP5$^+$ × PAO2376) for *catA* and its flanking markers. Linkage values are expressed as the percentage of selected recombinants which have coinherited the unselected marker. The number of recombinants tested ranged from 100 to 300. Arrowheads indicate the unselected markers. The figure is not drawn to scale. The numbers above the marker symbols refer to the map locations in Fig. 7.

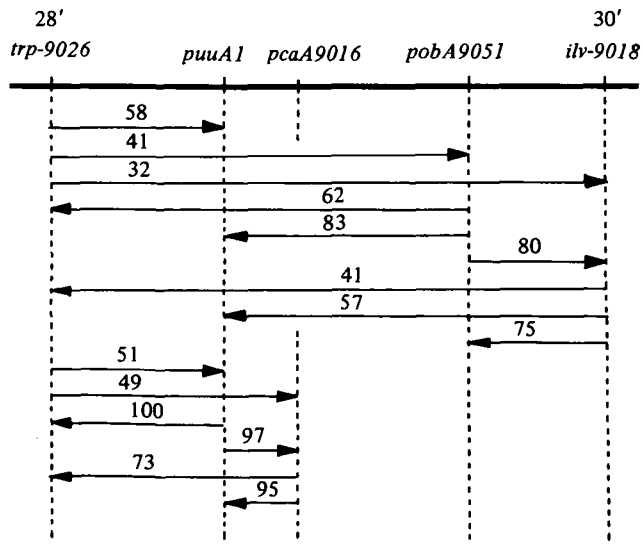

Fig. 2. Linkage values obtained from the matings (PAO2152, FP5$^+$ × PAO2374) and (PAO2152, FP5$^+$ × PAO2372) for *pobA* and *pcaA* and their flanking markers. Other details are as in the legend to Fig. 1.

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Hegeman (1968, 1971) and Rosenberg & Hegeman (1969). We have isolated *catA*, *pobA* and *pcaA* mutants (Table 2) and determined their chromosomal locations by linkage to already known markers using FP5-mediated crosses. The data from a variety of crosses is summarized in Figs. 1 and 2.

Table 3. *Growth response of* dcu *mutants to dicarboxylic acids and aromatic compounds*

Strain	Relevant mutation	Utilization of substrate as carbon source*						
		Glu	Adp (C6)	Pim (C7)	Sub (C8)	Aze (C9)	Ben	Pob
PAO2152	*dcu*$^+$	+	+	+	+	+	+	+
PAO4020	*dcu*$^+$	+	+	+	+	+	+	+
PAO4032	*dcu-9013, catA*	+	−	−	−	−	−	+
PAO4044	*dcu-9041, catA*	+	+	−	±	−	−	+
PAO4105	*dcu-9008*	+	+	−	+	−	+	+

+, growth occurred; ±, delayed, poor growth occurred; −, no growth.

* Abbreviation: Glu, glucose; Adp, adipate; Pim, pimelate; Sub, suberate; Aze, azelate; Ben, benzoate; Pob, *p*-hydroxybenzoate.

The *catA* gene was located in the late region of the chromosome and shown to be linked to *mtu-9002* of Royle *et al.* (1981) (Fig. 1). Genes *catA* and *mtu-9002* were shown by linkage analysis to be located between *tyu-9030* and *met-9020*.

Genes *pobA* and *pcaA* were previously known to be unlinked to *catA* (data not shown), and linkage analysis gave a map position for these markers between *trp-9026* and *ilv-9018* (Fig. 2).

Genes *puuA* and *pcaA* were found to be closely linked as shown in Fig. 2. This close linkage was confirmed by G101 transduction. When selection was made for *puuA*$^+$, *pcaA*$^+$ was 74% cotransducible, and when *pcaA*$^+$ was selected *puuA*$^+$ was 65% cotransducible. No cotransduction was found between *trp-9026* and *puuA*, *puuA* and *pobA*, and *pcaA* and *pobA*, respectively. The chromosomal location of *puuA* has already been reported (Matsumoto *et al.* 1978). The most likely order of the five markers as determined by the data shown in Fig. 2 is *trp-9026*, *puuA*, *pcaA*, *pobA* and *ilv-9018*.

(ii) dcu *mutants*

Utilization of the saturated dicarboxylic acids, adipate (C6), pimelate (C7), suberate (C8) and azelate (C9), by *P. aeruginosa* was reported by Stainier *et al.* (1966). Two kinds of *dcu* mutants differing in their growth response have been isolated (Table 3). PAO4032 (*dcu-9013*) has lost the ability to grow on all the dicarboxylic acids tested. The *dcu*$^+$ transconjugants of the mutant selected on pimelate-containing plates were able to grow on other dicarboxylic acids as well. PAO4044 (*dcu-9041*) retained the ability to grow on adipate and suberate, although growth on the latter was poor. Mutant PAO4105 (*dcu-9008*) had lost the ability to degrade dicarboxylic acids which have an odd number of carbon atoms (pimelate and azelate).

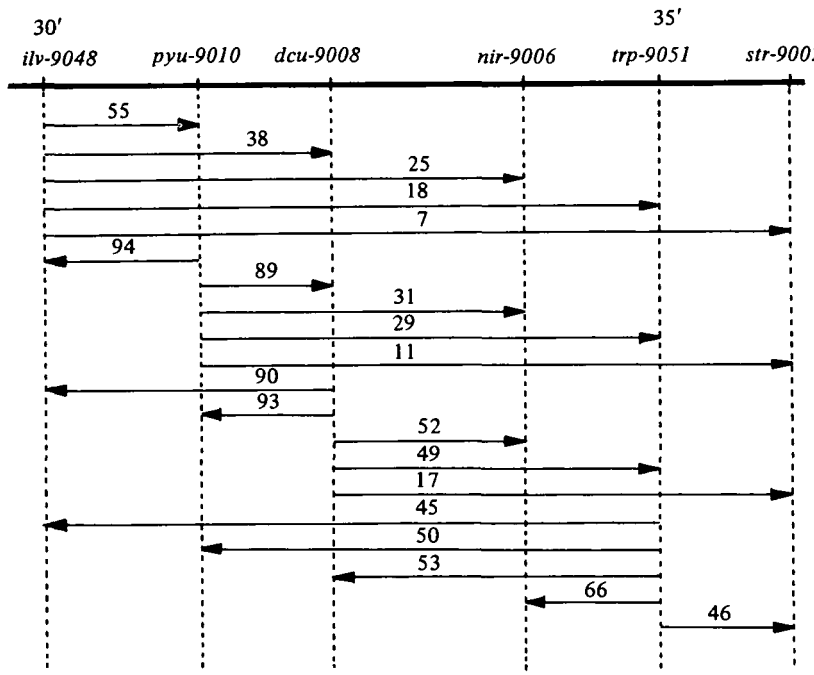

Fig. 3. Linkage values obtained from the mating (PAO4020, FP5$^+$ × PAO4105) for *dcu-9008* and its flanking markers. Other details are as in the legend to Fig. 1.

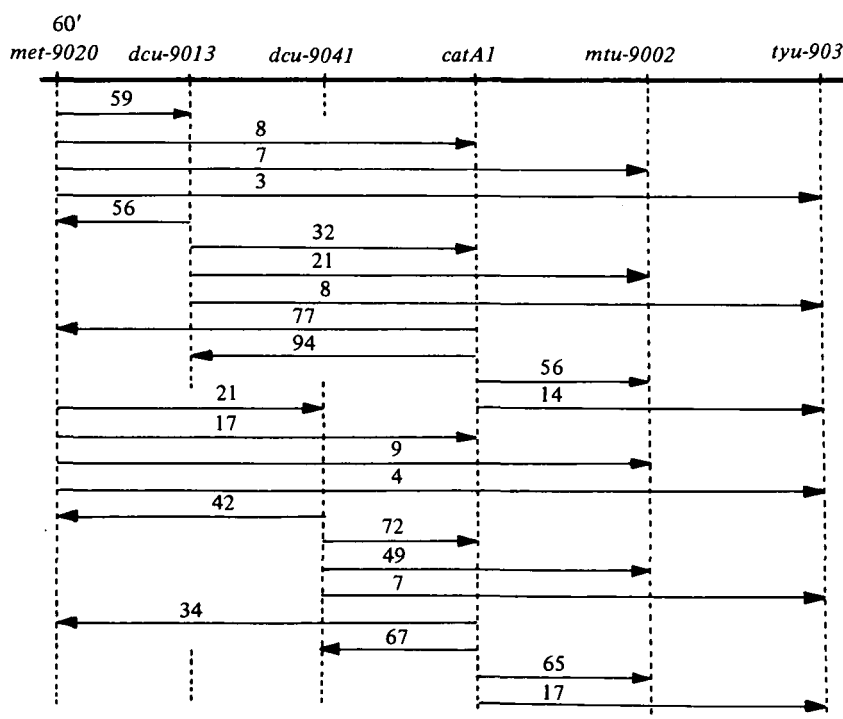

Fig. 4. Linkage values obtained from the matings (PAO2152, FP5$^+$ × PAO4032) and (PAO2152, FP5$^+$ × PAO4044) for *dcu-9013* and *dcu-9041* and their flanking markers. Other details are as in the legend to Fig. 1.

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The *dcu* mutations were found to lie in two different regions of the chromosome. In the mating between PAO2152 and PAO4105, we had no difficulty in isolating *dcu*$^+$ recombinants on selective plates containing 10^{-4} M tryptophan, isoleucine and valine. Linkage values for mutation *dcu-9008* indicated the relative order *ilv-9048*, *pyu-9010*, *dcu-9008*, *trp-9051* shown in Fig. 3. On the other hand, mutations *dcu-9013* and *dcu-9041* were mapped between *met-9020* and *catA* as shown in Fig. 4. We were unable to determine the order of the two *dcu* markers, *dcu-9013* and *dcu-9041*, relative to the flanking markers, *met-9020* and *catA*.

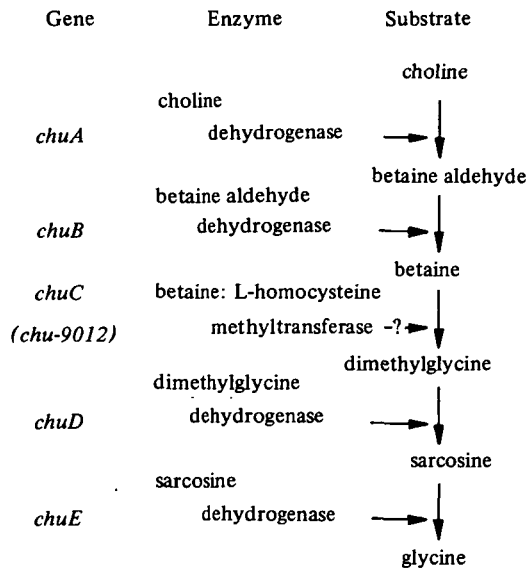

Fig. 5. Proposed degradative pathway of choline in *P. aeruginosa* PAO.

(iii) chu *mutants*

In *P. aeruginosa*, three enzymes which function in the catabolic pathway of choline to glycine, choline dehydrogenase, betaine aldehyde dehydrogenase and sarcosine dehydrogenase, have been described (Nagasawa *et al.* 1976*a*, *b*; Bater & Venables, 1977). We have isolated four kinds of *chu* mutants (Table 4). Eighteen mutants represented by PAO2389 (*chuA9003*) did not respond to choline but utilized all the other substrates tested and were shown to lack choline dehydrogenase. The growth response of the strains with *chu-9012* have led us to speculate that they might be deficient in betaine homocysteine methyltransferase activity, although this could not be confirmed by enzyme assays. Two groups of mutants, one represented by PAO2397 (*chuD9008*) and the other by PAO2194 (*chuE9002*) were identified as lacking dimethylglycine dehydrogenase (*chuD*) and sarcosine dehydrogenase (*chuE*), respectively. Their growth responses agreed with the corresponding enzyme deficiencies. The degradative pathway of choline in *P. aeruginosa* suggested by the biochemical characterization is presented in Fig. 5.

Table 4. *Growth responses and Enzyme activities of* chu *mutants*

Strain	Relevant mutation	Utilization of substrate*						Specific activity (units/mg)		
		Glu	Cho	Bet	Dmg	Sar	Gly	Choline dehydrogenase (Cho)†	Dimethylglycine dehydrogenase (Dmg)	Sarcosine dehydrogenase (Sar)
PAO2152	*chu*$^+$	+	+	+	+	+	+	1·28	0·39	6·38
PAO2364	*chu*$^+$	+	+	+	+	+	+	1·67	0·37	5·71
PAO2389	*chuA*	+	−	+	+	+	+	< 0·01	0·22	3·28
PAO2390	*chuA*, *chu-9051*	+	−	−	+	+	+	nd	0·26	6·00
PAO2391	*chuA*, *chuD*	+	−	−	−	+	+	nd	< 0·01	3·95
PAO2392	*chuA*, *chuE*	+	−	−	−	−	+	nd	0·19	< 0·01
PAO4010	*chu-9012*	+	−	−	+	+	+	1·90	0·24	5·01
PAO4016	*chu-9012*, *chuD*	+	−	−	−	+	+	nd	< 0·01	1·86
PAO4017	*chu-9012*, *chuE*	+	−	−	−	−	+	nd	0·30	< 0·01
PAO2397	*chuD*	+	−	−	−	+	+	2·24	< 0·01	3·39
PAO2194	*chuE*	+	−	−	−	−	+	0·71	0·35	0·04

+, growth occurred; −, no growth; nd, not determined.

* Abbreviation: Glu, glucose; Cho, choline; Bet, betaine; Dmg, dimethylglycine; Sar, sarcosine; Gly, glycine.

† Substrate shown in parentheses was added as an inducer for each enzyme.

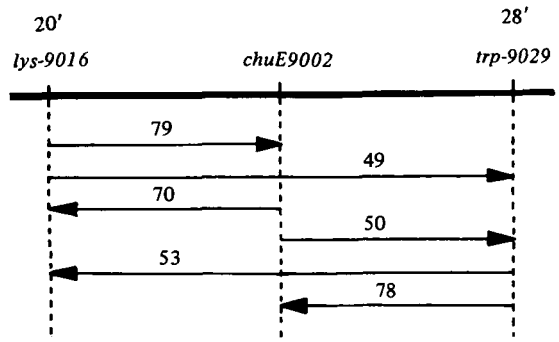

Fig. 6. Linkage values obtained from the mating (PAO2152, FP5^{+} × PAO2194) for *chuE* and its flanking markers. Other details are as in the legend to Fig. 1.

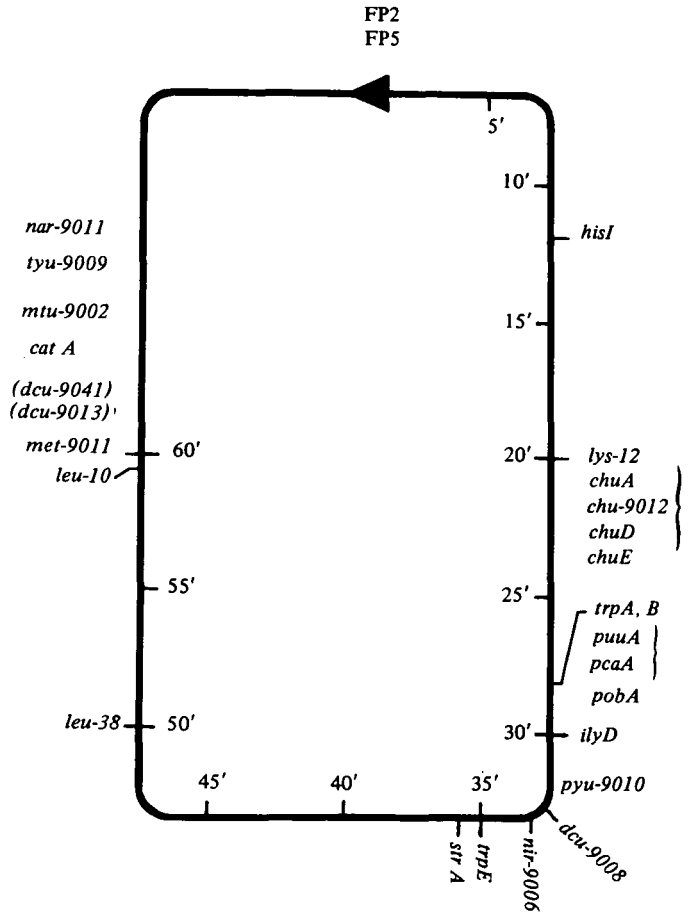

Fig. 7. Chromosomal location of *catA*, *pobA*, *pcaA*, *dcu* and *chu* genes of *P. aeruginosa* PAO relative to the previously mapped markers (Royle *et al.* 1981). The order of markers in parenthesis and that of the various *chu* genes relative to one another have not been determined. Brackets indicate that the markers are cotransducible. Marker abbreviations are listed in the footnote of Table 1.

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All the *chu* mutations showed linkage to *lys-9016* and *trp-9029* (and *trp-9031*) by conjugation, and the data for *chuE9002* are given in Fig. 6. The very close linkage of all the *chu* genes was confirmed by transduction using strains with double *chu* mutations (Table 5). The most likely order from these results is *chuA*, *chu-9012*, *chuD* and *chuE*.

(iv) *Chromosomal location of newly mapped genes*

The chromosomal locations of the eleven newly mapped genes obtained from the data given in Figs. 1, 2, 3, 4, 6 and Table 5 are shown in Fig. 7. Although interrupted mating data were not obtained for the various catabolic markers, reference markers with known map positions (Royle *et al.* 1981) have always been included in all the crosses. Therefore, it is possible to determine the positions of most of the new markers. The position of the *chu* gene cluster is in the 23–26 min region of the chromosome. The three closely linked markers, *puuA*, *pcaA* and *pobA*, lie in the 28–30 min region. The *dcu-9008* and *pyu-9010* may be situated on the 31–33 min region. We are unable to determine the positions of the three late markers, *dcu-9013*, *dcu-9041* and *catA*, since no marker has been accurately located beyond the *met-9020* (60 min).

4. DISCUSSION

Rosenberg & Hegeman (1969) and Kemp & Hegeman (1968, 1971) found that in *P. aeruginosa* the genes *catA*, *pobA* and *pcaA*, involved in the degradation of benzoate and *p*-hydroxybenzoate fell into three distinct groups. By conjugation we have shown that *pobA* and *pcaA* map near *trpA*, *B* and *ilvD*, while *catA* is located in the late region of the chromosome (Fig. 7). The wide distribution of these genes in *P. aeruginosa* is in marked contrast to the clustering of the corresponding genes in *Pseudomonas putida* (Wheelis, 1975). No linkage between the *dcu* genes and the *catA*, *pobA* or *pcaA* genes was demonstrated, although the dicarboxylic acids tested are known to be degraded *via* the β-ketoadipate pathway (Hoet & Stanier, 1970*a*, *b*).

The pathway of degradation of choline to glycine in *P. aeruginosa* shown in Fig. 5 seems to be the same as that found in mammalian cells (Jellinek *et al.* 1959; Frisell *et al.* 1962). We obtained mutants which appeared to be defective in each of the enzymes except betaine aldehyde dehydrogenase. In *Arthrobacter globiformis*, Ikuta *et al.* (1977) found that there were two enzymes, i.e. choline oxidase and betaine aldehyde dehydrogenase, both catalysing the conversion of betaine aldehyde to betaine. If this were also the case in *P. aeruginosa*, then it would be difficult to isolate mutants that are completely blocked at this step. The very close linkage of *chu* genes for degradation of choline to glycine is interesting, and it would be worthwhile to investigate if they constitute an operon.

Royle *et al.* (1981) have suggested that the location of anabolic and catabolic genes on the chromosome of *P. aeruginosa* may not be random and that most anabolic genes are found in the 'early region', while many genes participating in

Table 5. *Transductional analysis of* chuA, chu-9012, chuD *and* chuE *mutations*

Cross*	Mutation†				Selected marker	Substrate included in selective plate	Number of transductants tested	Frequency of cotransduction
	chuA	*chu-9012*	*chuD*	*ChuE*				
PAO1	+	+	+	+	·	·	·	·
PAO2390	*9003*	*9051*	+	+	*chu-9012*$^+$	betaine	315	*chuA* & *chu-9012*, 1·6 %
PAO2391	*9003*	+	*9052*	+	*chuD*$^+$	dimethylglycine	210	*chuA* & *chuD*, 1·0 %
PAO2392	*9003*	+	+	*9053*	*chuE*$^+$	sarcosine	315	*chuA* & *chuE*, < 0·3 %
PAO4016	+	*9012*	*9054*	+	*chuD*$^+$	dimethylglycine	315	*chu-9012* & *chuD*, 93 %
PAO4017	+	*9012*	+	*9055*	*chuE*$^+$	sarcosine	305	*chu-9012* & *chuE*, 90 %

* PAO1 was used as the donor in all the crosses.
† + indicates wild-type allele.

https://doi.org/10.1017/S0016672300020590 Published online by Cambridge University Press

the dissimilation of various compounds are located in the 'late region'. The present data suggested that catabolic genes may be more widely distributed than previously thought but further linkage analysis is needed. These newly mapped catabolic genes can serve as selective markers for the various chromosomal regions in which they are located and add to the precision of the chromosome map.

We are grateful to B. W. Holloway for his helpful advice and for reading this manuscript.

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