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Expression of the *argF* Gene of *Pseudomonas aeruginosa* in *Pseudomonas aeruginosa*, *Pseudomonas putida*, and *Escherichia coli*

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R′ plasmids carrying *argF* genes from *Pseudomonas aeruginosa* strains PAO and PAC were transferred to *Pseudomonas putida argF* and *Escherichia coli argF* strains. Expression in *P. putida* was similar to that in *P. aeruginosa* and was repressed by exogenous arginine. Expression in *E. coli* was 2 to 4% of that in *P. aeruginosa*. Exogenous arginine had no effect, and there were no significant differences between *argR*$^+$ and *argR* strains of *E. coli* in this respect.

The biosynthesis of amino acids in *Pseudomonas aeruginosa* and other *Pseudomonas* species follows pathways similar to those in *Escherichia coli*, but there is less clustering of genes, and few of the enzymes are regulated by repression of synthesis by the end products of the pathways (3, 4, 14). The arginine biosynthetic genes are scattered around the chromosome of *P. aeruginosa* PAO, and only *argF*, coding for biosynthetic ornithine carbamoyltransferase, is subject to severe repression by arginine (8). *P. aeruginosa* is able to utilize arginine as a carbon and nitrogen source for growth by more than one catabolic pathway (10). The arginine deiminase pathway (also known as the arginine dihydrolase pathway) is carried out by three specific enzymes induced by growth on arginine: arginine deiminase, catabolic ornithine carbamoyltransferase, and carbamate kinase. Growth with arginine also induces *N*-acetylornithine aminotransferase, which has transferase activities with glutamic semialdehyde and ornithine, so that this enzyme functions in both biosynthetic and catabolic pathways for arginine and ornithine (16). The two ornithine carbamoyltransferases are essentially unidirectional in their activities, and the catabolic ornithine carbamolytransferase cannot be used for arginine biosynthesis in *P. aeruginosa* strains with mutations in *argF*.

During the course of our studies on the amidase genes of *P. aeruginosa*, we found 20 to 30% linkage of *amiE* to *argF* and have obtained R′ plasmids carrying amidase genes by selecting for *argF* and screening for amidase activity. Previously, Hedges and Jacob (5) had isolated a plasmid carrying *argF* by selecting for prototrophic recombinants in a cross between *P. aeruginosa* PAC containing IncP-1 plasmid R68.44 and an arginine mutant of *E. coli*. At that time, the *E. coli* strain was thought to have a mutation in *argG*, but we have now shown that it is defective in *argF*. Holloway (7) isolated R′ *arg* plasmids by selection for conjugal transfer with R68.45 to recombination-deficient strains of *P. aeruginosa* PAO. Morgan carried out a similar selection but used auxotrophic mutants of *Pseudomonas putida* PPN as recipients (12). By this means, he selected a number of R′ plasmids containing chromosomal segments extending over several genes located near *argF* on the chromosome of strain PAO.

We have examined the expression of *argF* derived from either *P. aeruginosa* strain PAO or PAC in both these strains, in *P. putida* strain PPN, and in strains of *E. coli*. Simon Baumberg, University of Leeds, kindly provided us with strains of *E. coli* K-12 which carried *argF* deletions and were isogenic, except that some were *argR*$^+$ and others were *argR*. We were thus able to measure the expression of *P. aeruginosa argF* ornithine carbamoyltransferase in the related *Pseudomonas* strains and in *E. coli* and to test whether or not the product of the arginine regulatory gene *argR* from *E. coli* could act on *P. aeruginosa argF*.

The bacterial strains and plasmids used in this study are listed in Table 1. The stock cultures were maintained on nutrient or minimal salt agar stabs at room temperature. Carbenicillin (500 μg/ml) and kanamycin (250 μg/ml) were added for *Pseudomonas* strains carrying R′ plasmids, and 100 and 50 μg/ml, respectively, were added for *E. coli* strains. Plasmid pAR1 was maintained in *E. coli* W4100 *argF*. Plasmid pMO778 was maintained in *P. putida* PPN1022. Plasmid pAS13 was maintained in *P. putida* PPN1092 and was constructed as follows. *P. aeruginosa* PAO cultures harboring plasmid R68.45 had

TABLE 1. Bacterial strains and plasmids

Strain	Relevant genotype or phenotype[a]	Source/reference
P. aeruginosa		
PAO1	Wild-type ATCC 17503	B. W. Holloway
PAO29	*argF10 leu-10* FP2	B. W. Holloway
PAO127	*met-28*(pMO61)	B. W. Holloway
PAC1	Wild-type NCIB 10848	Laboratory stock (2)
PAC51	*argF504 amiE8*	This study
PAC21	*amiR11 lys-515*	This study
P. putida		
PPN1	Wild-type ATCC 12633	R. Y. Stanier
PPN1022	*argF402 cys-400 ilv-400 trpB402*	A. F. Morgan (12)
PPN1092	*argF407 met-406 trpF411*	A. F. Morgan (12)
E. coli		
K-12	Wild type	Laboratory stock
4100	*argI thi*	N. Glansdorff
4100 Arg	*argF argI thi*	R. W. Hedges (4)
SC1800	Δ(*proAB-argF-lac*)XIII *his metB argI90 mal xyl* (*argR*$^+$)	S. Baumberg
SC1800 RH1	As above but *argR*	S. Baumberg
SC1800 RH2	As above but *argR*	S. Baumberg
Plasmids		
pMO61	Cb^r Km^r Tc^r	A. F. Morgan
pMO778	Cb^r Km^r *argF* from PAO	A. F. Morgan
pAR1	Cb^r Km^r *argF* from PAC	R. W. Hedges (5)
pAS13	Cb^r Km^r *argF amiRE* from PAC	This study

[a] Genotype symbols are as used by Bachmann and Low (1) for *E. coli* with the addition of *amiRE* for the amidase regulator and structural genes (2). The phenotype abbreviations are for resistance to carbenicillin, kanamycin, and tetracycline.

been found to give rise to variants with enhanced chromosome-mobilizing ability and increased ability to generate R′ plasmids in crosses with *P. putida* (12; A. F. Morgan, personal communication). *P. aeruginosa* PAO127(pMO61) was crossed with PAC21 (*amiR11 lys-515*), and selection was made for transfer of carbenicillin and kanamycin resistance. PAC21(pMO61) was then used as a donor in a cross with PPN1092 (*trpF met argF*) with selection for arginine prototrophy as described by Morgan (12). The transconjugants were tested for the ability to grow on acetamide, and 2% of those acquiring *argF* were found also to have acquired *amiE*. One of these was pAS13. The PPN1092 strains carrying R′ plasmids were checked for growth phenotype, and the plasmids were transferred into *P. aeruginosa* test strains by plate mating. Transfers of plasmids from *P. putida* to *E. coli* were carried out by plate matings in which the spread plates were incubated at 30°C for 4 h and then transferred to 37°C.

Nutrient broth was prepared from Oxoid nutrient broth no. 2 (Oxoid Ltd., London, England) and Bacto-Agar (Difco Laboratories, Detroit, Mich.) added for slopes and plates. The *Pseudomonas* strains were grown in minimal salt medium with 0.5% sodium succinate (2). The *E. coli* strains were grown in minimal salt medium, devised by J. H. Marshall, which had the following composition in 1 liter: Na_2HPO_4, 4.5 g; KH_2PO_4, 2 g; $(NH_4)_2SO_4$, 1 g; NH_4Cl, 1 g; KNO_3, 0.5 g; 10 ml of trace element solution; 0.2% glucose or 0.2% glucose plus 0.5% lactate as the carbon source. The appropriate amino acid and growth factor supplements were added to give final concentrations of: cystine, 0.05 mM; thiamine, 5 μM; isoleucine, valine, histidine, tryptophan, and methionine, 0.25 mM. Cultures for enzyme studies were grown for 16 to 18 h in 300 ml of medium contained in conical 3-liter flasks and incubated by shaking on a reciprocating shaker. *P. aeruginosa* and *E. coli* strains were grown at 37°C, and *P. putida* strains were grown at 30°C. Cultures were harvested by centrifugation at 6,000 × *g* for 10 min and washed with Tris-saline buffer (0.145 M NaCl in 0.35 M Tris-hydrochloride; pH 8.5). The bacterial pellets were kept at −20°C until required. Before use, the pellets were suspended to give 10 mg (dry weight) of bacteria per ml, kept ice-cold, and disrupted in a semiautomatic French pressure cell (Aminco, Silver Spring, Md). Ornithine carbamoyltransferase activity was mea-

TABLE 2. Repression of ornithine carbamoyltransferase by arginine in strains with wild-type *argF* and *argR* genes[a]

Strain	Ornithine carbamoyltransferase activities		% Repression
	No arginine	5 mM arginine	
E. coli K-12	47	1.6	97
E. coli 4100	28	0.9	96
P. aeruginosa PAO1	23	3.5	84
P. aeruginosa PAO1	23	8	65
P. putida PPN1	16	7.5	53

[a] Cultures were grown at 37°C for 17 to 18 h, at which time they had reached an absorbance at 670 nm of about 1.0. The specific activities are expressed in micromoles of citrulline produced per hour per milligram of protein.

sured by the method of Stalon et al. (15). The final volume of the reaction mixture was 1 ml and contained about 1 mg of protein. Citrulline determinations were made with 10- to 200-μl samples of the reaction mixture after removal of precipitated protein by centrifugation. Reagent blanks and zero-time blanks were included in each set of assays, but no citrulline was detected in any of them. All the citrulline measurements were made with an amino acid analyzer (Locarte, London, England) as described previously (13). All enzyme assays were carried out in duplicate, and duplicate determinations of citrulline were made from each of the assay mixtures. This method allowed very low enzyme activities to be measured since less than 1 nmol of citrulline could be detected by this means. Protein was routinely determined by the method of Lowry (9), but rapid determinations were made by measuring the absorbance at 260 and 280 nm with an SP8 200 spectrophotometer (Pye Unicam, Cambridge, England).

The phenotype of the presumed *argF* mutants was as expected. All grew on minimal agar plates supplemented with arginine or citrulline, but not with ornithine. Strain 4100 *arg* behaved in the same way as the known *argF* mutants, thus confirming that this strain was defective in *argF* and also lacked the alternative ornithine carbamoyltransferase gene, *argI*. Table 2 shows the effect of exogenous arginine on ornithine carbamoyltransferase in wild-type *Pseudomonas* and *E. coli* strains. The *E. coli* strains showed strong repression by 5 mM arginine. The enzymes of *P. aeruginosa* and *P. putida* were also repressed by arginine, although the repression in *P. putida* appeared to be less severe than in *P. aeruginosa* in these experiments. Isaac and Holloway (8) had found that the level of the biosynthetic ornithine carbamoyltransferase of *P. aeruginosa* varies with the growth rate and is repressed about fivefold by 5 mM arginine. For glycerol and glutamate minimal medium, they found specific activities of 28 and 23, respectively.

The two *P. aeruginosa* strains with *argF* mutations synthesized high levels of ornithine carbamoyltransferase when carrying R′ *argF* plasmids derived from either of the two strains (Table 3). It appeared that repression by arginine of the plasmid-carried *argF* was more marked in strain PAC than in PAO. *P. putida* produced lower activities in the wild-type strain, and also had lower activities when it carried one of the two *P. aeruginosa* R′ plasmids, than either of the *P. aeruginosa* strains. The *argF* expression was well above the threshold needed for growth, and the strains carrying plasmids grew as well as those with wild-type *P. putida argF* genes.

The expression of *P. aeruginosa argF* in *E. coli* was poor (Table 4), and this is in agreement with results obtained for other biosynthetic genes. R′ *trpAB* plasmids isolated and studied by Hedges et al. (6) were able to complement tryptophan auxotrophs of *E. coli*, but the transconjugants grew poorly and enzyme levels were very low. In our experiments, the amount of ornithine carbamoyltransferase was 2 to 4% of that produced in the parental *P. aeruginosa* strain (Table 4). These *E. coli* plasmid-carrying strains also grew rather slowly. The lag period was prolonged, and in the absence of added arginine, the strains carrying R′ *argF* plasmids took 24 h to reach the end of the exponential

TABLE 3. Expression of *P. aeruginosa* R′ *argF* plasmids in *Pseudomonas* strains[a]

Strain	Plasmid	Ornithine carbamoyltransferase activity		% Repression
		No arginine	5 mM arginine	
PAO29	None	NG	0	
	pAR1	70	34	52
	pMO778	103	48.5	53
PAC51	None	NG	0	
	pAR1	26	7	73
	pMO778	77.5	22	72
PPN1022	None	NG	0	
	pAR1	15.5	4	74
	pMO778	17	9	47

[a] Cultures were grown at 37°C for 17 to 18 h, giving an absorbance at 670 nm of about 1. Growth conditions and enzyme activities are described in Table 2. NG, No growth.

TABLE 4. Expression of *P. aeruginosa* R' *argF* plasmids in *E. coli argF* strains[a]

Strain	Plasmid	Ornithine carbamoyltransferase activity	
		No arginine	5 mM arginine
4100 *argF*	None	NG	0.03
	pAR1	0.7 (0.6–0.9)	0.5 (0.3–0.8)
	pMO778	0.7 (0.4–1.0)	0.4 (0.2–0.6)
SC1800 *argR*$^+$	None	NG	<0.01
	pAR1	0.9 (0.6–1.2)	0.4 (0.3–0.4)
	pMO778	1.1 (0.4–1.9)	0.4 (0.4)
	pAS13	1.1 (0.6–1.7)	0.4 (0.4–0.6)
SC1800 RH1 *argR*	None	NG	<0.01
	pAR1	0.5 (0.4–0.6)	0.4 (0.4)
	pMO778	0.8 (0.65–0.9)	0.6 (0.4–0.7)
	pAS13	1.0 (0.8–1.4)	0.5 (0.4–0.7)
SC1800 RH2 *argR*	None	NG	<0.01
	pAR1	0.65 (0.6–0.7)	0.6 (0.3–0.8)
	pMO778	0.7 (0.5–0.9)	0.6 (0.3–0.8)
	pAS13	1.1 (0.6–1.5)	0.5 (0.3–0.6)

[a] Cultures were grown until the absorbance at 670 nm reached about 1. The *argF* strains grown without arginine took up to 24 h and with arginine took 17 to 18 h. The specific activities are given as the mean of four or five separate growth experiments with the range of values shown in parentheses. NG, No growth.

growth phase. It appeared at first from the results obtained with *E. coli* 4100 *argF* carrying either pAR1 or pMO778 that there might be some repression exerted by the *E. coli argR* product on the *P. aeruginosa argF* gene. When the *argF* plasmids were transferred to the *argF* strains constructed by S. Baumberg we found that they produced ornithine carbamoyltransferase activities similar to those found with strain 4100 *argF*(pAR1) (and other *E. coli* strains not shown here). However, there were no significant differences in expression of *P. aeruginosa argF* between those *E. coli* strains that carried the *argR*$^+$ allele and the strains with *argR* mutations. The differences in the enzyme levels of cultures growing with or without arginine could be ascribed to differences in growth rate. At this level of expression, the activity of ornithine carbamoyltransferase is rate-limiting for growth, and the cultures presumably have very depleted arginine pools. When arginine at 5 mM is added to the minimal medium, the growth rate increases and the enzyme activity falls to an even lower level.

Mergeay et al. (11) found that the *argE* and *argH* products from *E. coli* genes were expressed in *Pseudomonas fluorescens* at values close to those for wild-type *E. coli*. This suggested that there is no serious limitation to the expression of *E. coli* genes in *P. fluorescens*, even though the expression of *P. aeruginosa argF* and *trpAB* in *E. coli* is at a reduced level. The regulation of biosynthetic genes is different, and in *P. aeruginosa* the *trpAB* genes are not repressed by tryptophan but are induced by indole glycerol phosphate. *E. coli trpA* (*ptrpAB*) exhibited the *Pseudomonas* type of regulation (6). *P. aeruginosa arg* genes, with the exception of *argF*, are not repressed by arginine (8), and Mergeay et al. (11) found that exogenous arginine has no effect on the expression of *E. coli argEH* in *P. fluorescens*. We have now shown that *P. aeruginosa argF*, which is severely repressed by exogenous arginine in *P. putida* and *P. aeruginosa*, is not repressed by arginine in *E. coli* and that the *E. coli argR* product is unable to exert regulatory control.

The ways in which RNA polymerase-promoter interactions allow *E. coli* genes to be expressed more efficiently in *P. fluorescens* than *P. aeruginosa* genes are expressed in *E. coli*, and the detailed interactions of the regulator gene products, remain to be elucidated by in vitro studies.

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