The *arcABC* Operon Required for Fermentative Growth of *Pseudomonas aeruginosa* on Arginine: Tn5-751-assisted Cloning and Localization of Structural Genes

By ERNST LÜTHI,¹ ANNICK MERCENIER² AND DIETER HAAS^{1*}

¹Mikrobiologisches Institut, Eidgenössische Technische Hochschule, CH-8092 Zürich, Switzerland

²Transgène SA, F-67000 Strasbourg, France

(Received 14 April 1986; revised 2 June 1986)

Pseudomonas aeruginosa is able to utilize L-arginine as the energy source for growth under anaerobic, nitrate-free conditions. Mutations in the chromosomal *arcABC* gene cluster specifying the inducible arginine deiminase pathway enzymes abolish fermentative growth on arginine. From two different *arc*:Tn5-751 insertion mutants of *P. aeruginosa* recombinant plasmids have been derived which carry a resistance marker of transposon Tn5-751 plus flanking parts of the *arc* region. These recombinant plasmids served to reconstruct *in vitro* the functional *arcABC* cluster on a 5.6 kb fragment, which was inserted into the broad-host-range vector pKT240. In *P. aeruginosa* this 5.6 kb segment complemented *arcABC* mutations in *trans* and contained the control region necessary in *cis* for *arc* enzyme induction by oxygen limitation and arginine. The results of subcloning experiments and transcriptional *lacZ* fusions, the polarity of transposon insertions and the effect of external promoters led to the conclusion that the structural genes *arcA* (for arginine deiminase), *arcB* (for catabolic ornithine carbamoyltransferase) and *arcC* (for carbamate kinase) are contiguous and transcribed in the same direction. Thus, the *arcABC* cluster appears to have the characteristics of an operon. In *Escherichia coli* the cloned *arcABC* genes were expressed at low, non-inducible levels; strong vector promoters enhanced *arc* expression up to 100-fold. This indicates that transcriptional initiation at the *arc* promoter(s) is poor in *E. coli*.

INTRODUCTION

Pseudomonas aeruginosa is usually considered a strict aerobe. It can use nitrate instead of oxygen as a terminal electron acceptor for anaerobic growth (Palleroni, 1984). When oxygen and nitrate are absent, *P. aeruginosa* is capable of slow growth in rich medium with L-arginine as the sole energy source (Vander Wauven *et al.*, 1984). Mutants that cannot grow by fermentation of arginine (*arc* mutants) are affected in the enzymes of the arginine deiminase pathway or in unknown accessory functions. The arginine deiminase pathway consists of three enzymes: arginine deiminase (ADI), catabolic ornithine carbamoyltransferase (cOTC), and carbamate kinase (CK), which are the products of the chromosomal *arcA*, *arcB*, and *arcC* genes, respectively (Mercenier *et al.*, 1982; Vander Wauven *et al.*, 1984). These enzymes convert L-arginine to L-ornithine and generate 1 mol ATP per mol arginine. Oxygen limitation and arginine together produce strong coordinate induction of the deiminase pathway enzymes; conditions of energy depletion also have an inductive effect (Mercenier *et al.*, 1980). Under aerobic conditions, the *arc* mutants are able to utilize L-arginine normally as the C and N source via other pathways (Haas *et al.*, 1984a).

Abbreviations: ADI, arginine deiminase; cOTC, catabolic ornithine carbamoyltransferase; CK, carbamate kinase; OS, minimal medium; YEA, yeast extract/arginine medium.

Catabolic enzymes acting as a functional unit are often specified by clustered genes in *Pseudomonas* (Haas, 1983). The gene arrangements of several degradative *Pseudomonas* plasmids have been studied in molecular detail. Much less is known about the organization of chromosomal genes in *Pseudomonas* (Holloway, 1984). Previously, we have shown that the *arcA*, *arcB*, and *arcC* genes are highly cotransducible (Vander Wauven *et al.*, 1984) and that transposon Tn5-751 insertions causing an Arc⁻ phenotype are clustered in a chromosome segment of about 3 kb (Haas *et al.*, 1984b; Rella *et al.*, 1985). The *arc* genes adjacent to the Tn5-751 insertions have been isolated on recombinant plasmids with *Escherichia coli* as the host (Rella *et al.*, 1985). We have now used two of these hybrid plasmids to reconstruct *in vitro* the entire *arcABC* cluster. The cloned *arcABC* genes appear to be contiguous and organized as an operon; their regulation by oxygen limitation and arginine was observed in *P. aeruginosa* but not in *E. coli*.

METHODS

Bacterial strains and plasmids. They are listed in Table 1. Plasmids constructed in this study are shown in Figs 1 and 2. Recombination-deficient (*rec-102*) derivatives of *arc* mutants were constructed by the method of Früh *et al.* (1983).

Media and culture conditions. The complete yeast extract/arginine (YEA) medium and the minimal (OS) glutamate/arginine medium for anaerobic or oxygen-limited growth of *P. aeruginosa* have been described (Vander Wauven *et al.*, 1984). The media and antibiotic concentrations used in genetic experiments were the same as previously given (Itoh *et al.*, 1984; Haas *et al.*, 1984a; Jeenes *et al.*, 1986). *P. aeruginosa* cultures were grown at 37 °C on a rotary shaker (180 r.p.m.). Good aeration was achieved in baffled Erlenmeyer flasks; cells were collected in the exponential phase. Oxygen limitation was obtained in hermetically closed 125 ml bottles containing 60 ml medium; after an initial phase of aerobic growth cells were depleted of oxygen and harvested in the stationary phase after 36 h incubation. Complementation tests of *arc* mutants were done on YEA plates incubated in a GasPak anaerobic jar (Vander Wauven *et al.*, 1984).

Enzyme assays. *E. coli* extracts were prepared by sonication and *P. aeruginosa* cells were permeabilized with toluene for enzyme assays as described by Haas *et al.* (1979). ADI (EC 3.5.3.6) was measured in 1.0 ml 0.1 M-sodium citrate buffer pH 5.5 with 10 mM-L-arginine. After the reaction, 1.0 ml 1 M-HCl and 1.0 ml colour reagent [3% (w/v) diacetylmonoxime in ethanol/H₃PO₄/H₂SO₄, 2:9:3, by vol.] were added and citrulline production was determined as previously described (Haas *et al.*, 1979). The assays for cOTC (EC 2.1.3.3) and CK (EC 2.7.2.2) were done by published methods (Haas *et al.*, 1979; Mercenier *et al.*, 1980). Enzyme specific activities fluctuated by a factor of ≤ 2 in individual experiments; in induced cells, the fluctuations were mainly due to differential enzyme stability and variations in the cellular energy state at harvest. For ADI, cOTC and CK, 1 unit is defined as the amount of enzyme forming 1 μ mol product h⁻¹. β -Galactosidase (EC 3.2.1.23) activity was measured in arbitrary units defined by Miller (1972). Protein concentrations were determined by a modified Lowry procedure (Vander Wauven *et al.*, 1984).

DNA manipulations. Plasmid DNA was purified as described by Itoh *et al.* (1984). Small-scale plasmid preparations were obtained by the rapid boiling method (Holmes & Quigley, 1981), followed by phenol and chloroform extraction. In subcloning experiments DNA fragments were purified from agarose gels essentially by the method of Dretzen *et al.* (1981); instead of DEAE paper the NA-45 DEAE membrane (Schleicher & Schuell) was used. DNA fragments were eluted from the membrane by 250 μ l high-salt buffer (1 M-NaCl, 20 mM-Tris/HCl pH 8.0, 0.1 mM-EDTA) at 65 °C for 1 h. The membrane was washed with a further 50 μ l high-salt buffer and the eluate was centrifuged for 15 min. The supernatant was diluted to 0.5 M-NaCl and passed through a 100 μ l DEAE-cellulose column, which was washed with 3 $\times$ 1 ml column buffer (20 mM-Tris/HCl pH 8.0, 2.5 mM-EDTA). DNA fragments were eluted with 800 μ l column buffer containing 2 M-NaCl and recovered by ethanol precipitation. Conditions for restriction, ligation, 'fill-in' reaction with Klenow DNA polymerase I, and transformation of *E. coli* and *P. aeruginosa*, have been given (Itoh *et al.*, 1984). All cloning experiments were done in *E. coli* strains (Table 1). LacZ⁺ constructs were visualized on X-Gal (5-bromo-4-chloro-3-indoxyl- β -D-galactoside) nutrient agar (Miller, 1972).

Construction of Arc⁺ recombinant plasmids and their introduction into *P. aeruginosa*. An R68.45'HisII⁺Arc⁺ plasmid (our unpublished results) was partially digested with *Xho*I and ligated with *Xho*I-cut pKT240, giving pME100 (Fig. 2). All other Arc⁺ recombinant plasmids were derived from pME163 (Haas *et al.*, 1984b) and pME171 (Rella *et al.*, 1985) via pME180 or pME183, using *E. coli* as the host (Fig. 1). The pKT240Arc⁺ recombinants to be tested in *P. aeruginosa* were transferred to 43 °C-grown PAO and PTO strains by transformation (Itoh *et al.*, 1984) or mobilization (Jeenes *et al.*, 1986).

Table 1. Bacterial strains and plasmids

<i>P. aeruginosa</i> strain	Genotype	Reference or construction
PAO1	Wild-type (<i>cml</i> -2)	Holloway (1984)
PAO6091	<i>met</i> -9011 <i>ami</i> E200 <i>strA</i> <i>arc</i> -6091 :: Tn5-751	Haas <i>et al.</i> (1984b)
PAO6094	<i>met</i> -9011 <i>ami</i> E200 <i>strA</i> <i>arc</i> A6094 :: Tn5-751	Rella <i>et al.</i> (1985)
PAO6095	<i>met</i> -9011 <i>ami</i> E200 <i>strA</i> <i>arc</i> B6095 :: Tn5-751	Rella <i>et al.</i> (1985)
PAO6096	<i>met</i> -9011 <i>ami</i> E200 <i>strA</i> <i>arc</i> -6096 :: Tn5-751	Rella <i>et al.</i> (1985)
PAO6138	<i>met</i> -9011 <i>ami</i> E200 <i>arc</i> D6138	Vander Wauven <i>et al.</i> (1984)
PTO66	<i>his</i> -4 <i>lys</i> -12 <i>ilv</i> -118 <i>trp</i> -6 <i>pro</i> -82 <i>rec</i> -102	Früh <i>et al.</i> (1983)
PTO6091	<i>arc</i> -6091 :: Tn5-751 <i>strA</i> <i>rec</i> -102	Met ⁺ Ami ⁺ Rec ⁻ recombinant of PTO66(R68.45) × PAO6091*
PTO6095	<i>arc</i> B6095 :: Tn5-751 <i>strA</i> <i>rec</i> -102	Met ⁺ Ami ⁺ Rec ⁻ recombinant of PTO66(R68.45) × PAO6095*
PTO6096	<i>arc</i> -6096 :: Tn5-751 <i>strA</i> <i>rec</i> -102	Met ⁺ Ami ⁺ Rec ⁻ recombinant of PTO66(R68.45) × PAO6096*
<i>E. coli</i> strain	Genotype	Source or reference
CM236	Δ(<i>lac proAB argF</i>) Δ(<i>argI</i>) <i>thi</i> -1 <i>supE</i> <i>recA</i> 1 <i>srl</i> :: Tn10 <i>r</i> _K <i>m</i> _K	M. Faelen
HB101	<i>hsd</i> S20 <i>recA</i> 13 <i>proA</i> 2 <i>leu</i> -6 <i>thi</i> -1 <i>rps</i> L20 <i>ara</i> -14 <i>gal</i> K2 <i>lac</i> Y1 <i>xyl</i> -5 <i>mtl</i> -1 <i>supE</i> 44	N. Willetts
JM105	<i>thi</i> <i>rps</i> L <i>endA</i> <i>sbc</i> B15 <i>hsd</i> R4 Δ(<i>lac proAB</i>)/F' <i>tra</i> D36 <i>proAB</i> ⁺ <i>lac</i> ^a ZΔM15	Yanisch-Perron <i>et al.</i> (1985)
ED8654	<i>metB</i> <i>supE</i> <i>supF</i> <i>hsd</i> R	Borck <i>et al.</i> (1976)
Plasmid	Relevant characteristics	Source or reference
pBR322	Ap/Cb Tc	J. M. Watson
pME481	Ap/Cb (pBR322ΔTc)	Rella <i>et al.</i> (1985)
ColE1 :: Tn5 <i>lac</i>	Km <i>lacZYA</i> ⁺ (ColE1 with promoter probe Tn5 derivative)	Kroos & Kaiser (1984)
pJF118EH	Ap/Cb <i>lacI</i> ^a (expression vector with <i>tac</i> promoter)	E. Lanka
pNEO	Ap/Cb Km/Nm (expression vector with Tn5 Kan promoter)	Pharmacia PL Biochemicals
pKT240	Ap/Cb Km Sm (IncP-4 broad-host- range vector)	Bagdasarian <i>et al.</i> (1983)
pME163	Ap/Cb Tp ArcA ⁺ B ⁺ C [±]	Haas <i>et al.</i> (1984b)
pME171	Ap/Cb Tp ArcA ⁻ B ⁻ C ⁻	Rella <i>et al.</i> (1985)
pME100, 183, 188, 190, 191, 192, 197, 235, 239, 244; pKT240 <i>lacZ</i>	} pKT240 derivatives	Figs 1 and 2
pME177, 180		
pME179, 185, 186, 187, 195	} pBR322 derivatives	Fig. 2
pME246		
	pJF118EH derivative	Fig. 2

* Selection for Met⁺ allowed the introduction of the *rec*-102 marker, which is linked to *met*-9011 and *ami*E (Früh *et al.*, 1983).

RESULTS

Characterization of Tn5-751 insertions in chromosomal *arc* genes

In previous work (Haas *et al.*, 1984b; Rella *et al.*, 1985) four Tn5-751 insertions causing an Arc⁻ phenotype have been mapped physically on the *P. aeruginosa* chromosome (Fig. 1). We now examine their effect on the enzymes of the ADI pathway (Table 2). The transposon insertion in strain PTO6091 pleiotropically affected all three deiminase pathway enzymes, which became virtually non-inducible by oxygen limitation. The insertion in strain PAO6094

Fig. 1. Effect of chromosomal Tn5-751 insertions on Arc phenotype of *P. aeruginosa* and reconstruction of the functional *arcABC* operon *in vitro*. The plasmids pME163 and pME171 were derived from strains PAO6091 and PAO6094, respectively, by cloning the trimethoprim resistance gene (Tp^R) of Tn5-751 (Δ) plus flanking *arc* sequences (Haas *et al.*, 1984b; Rella *et al.*, 1985). The location of *arcA*, *arcB*, and *arcC* is taken from Fig. 2. Reconstruction of the *arcABC* operon was done in three steps (heavy arrows, 1-3), as described in the text. Double lines, chromosomal *P. aeruginosa* DNA; single lines, Tn5-751 DNA (IS50R and Tp^R); dashed lines, vector plasmid DNA. The dotted area contains the *arc* operon. The *Sma*I site in the dotted area is unique; other *Sma*I sites outside *arc* are not shown.

inactivated the *arcA* gene and had a weak polar effect on *arcB*. The mutation in strain PTO6095 inactivated *arcB* and was strongly polar on *arcC*. The transposon insertion in strain PTO6096 appears to be outside the *arc* structural genes but had a polar effect on the induced levels of the *arcABC* gene products. The polarity of the Tn5-751 insertions suggests that the gene order is *arcA-arcB-arcC*; subcloning experiments (see below) supported this finding.

Reconstruction *in vitro* of the *arcABC* cluster

Tn5-751 carries determinants for kanamycin and trimethoprim resistance, with a unique *Eco*RI site between the two resistance markers (Rella *et al.*, 1985). Chromosomal *Eco*RI

Table 2. Regulation of the *arc* operon by oxygen limitation in the presence of arginine

Cells were grown in YEA medium with good (+O₂) or limiting (−O₂) aeration and assayed for the *arc* enzymes. Strains carrying pME183 were tested once; the other values are means of two or three determinations. Note that the PTO strains are Rec[−] derivatives of the corresponding PAO strains.

Strain	Specific enzyme activity [units (mg protein) ^{−1}]					
	ADI		cOTC		CK	
	+O ₂	−O ₂	+O ₂	−O ₂	+O ₂	−O ₂
PAO1	3	54	60	975	80	525
PTO6091	0.2	0.6	13	23	80	165
PAO6094	<0.1	<0.1	16	240	NT	790
PTO6095	0.7	25	<5	<5	NT	<10
PTO6096	10	8	140	240	NT	250
PTO6091/pME183	23	410	630	7200	NT	1470
PAO6094/pME183	7	360	300	5400	NT	2100
PTO6095/pME183	31	330	450	6400	NT	1300
PTO6096/pME183	25	270	560	5200	NT	2200

NT, Not tested.

Table 3. Regulation of the *arc* operon by arginine during oxygen limitation

Cells were grown in minimal medium OS + 25 mM-L-glutamate with (+Arg) or without (−Arg) 25 mM-L-arginine under conditions of limited aeration. Enzyme assays were done in duplicate.

Strain	Specific enzyme activity [units (mg protein) ^{−1}]			
	ADI		cOTC	
	− Arg	+ Arg	− Arg	+ Arg
PAO1	30	67	370	1100
PTO6095	9	26	NT	NT
PTO6095/pME183	21	125	460	1400

NT, Not tested.

fragments from strains PAO6091 and PAO6094 have been cloned in *E. coli* with selection for trimethoprim resistance; in the recombinant plasmids pME163 and pME171 (Haas *et al.*, 1984*b*; Rella *et al.*, 1985; see Fig. 1) the trimethoprim resistance marker is flanked by *arc* fragments, which, however, were defective in *arc* enzyme expression (data not shown). We reconstructed a functional *arcABC* cluster by sequentially cloning the chromosomal 7.1 kb *Bgl*II fragment of pME163 and the chromosomal 5.6 kb *Bgl*II fragment of pME171 into the *E. coli* vector pNEO, resulting in the ArcA⁺B⁺C⁺ recombinant pME180 (steps 1 and 2 in Fig. 1). In particular, the correct orientation of both *Bgl*II fragments in the reconstruction restored the *arcA* function. Subcloning of the central 5.6 kb *Hind*III–*Cla*I fragment from pME180 into the broad-host-range vector pKT240 cleaved with *Hind*III + *Cla*I (step 3 in Fig. 1) gave the recombinant pME183, which complemented the four *arc* : : Tn5-751 mutants of *P. aeruginosa* for anaerobic growth on arginine.

In *P. aeruginosa*, the *arc* enzymes encoded by pME183 were inducible by oxygen limitation (Table 2) and by the addition of L-arginine to the growth medium (Table 3). Expression of the chromosomal *arc* genes is similar (Mercenier *et al.*, 1980; see Tables 2 and 3). In *E. coli*, by contrast, pME183 specified very low *arc* enzyme levels (Fig. 2, top line), which were not regulated by energy depletion and arginine (data not shown). Thus, pME183 carries the control region needed for regulation of the *arcABC* genes in *P. aeruginosa* but appears to lack regulatory elements that would allow regulated *arc* enzyme expression in *E. coli*.

Fig. 2. Localization of the *arcABC* structural genes and determination of the direction of transcription. All recombinant plasmids consisted of the vector plasmids indicated and the inserts of chromosomal DNA shown. The only exception is pME100, which carried an extra random 3.8 kb chromosomal *XhoI* fragment upstream of *arcABC* (not shown). All plasmids had a selectable ampicillin/carbenicillin resistance determinant. Vector promoters (◁) were: P_{Neo}, from the Tn5 neomycin/kanamycin resistance (via plasmid pNEO); P_{Kan}, from the Tn903 kanamycin resistance (on pKT240); P_{Tet}, from the tetracycline resistance of pBR322; P_{Tac}, hybrid *trp-lac* promoter, regulated by the *lacI* repressor (Bagdasarian *et al.*, 1983). The *lacZ* promoter probe fragment (4.1 kb) was derived from Tn5*lac* (Kroos & Kaiser, 1984).

* CK could not be measured accurately because of high blank activities: ++, activity > 20; +, activity < 20; -, no detectable activity; NT, not tested. IPTG, isopropyl-β-D-thiogalactoside added at 1 mM to exponentially growing cells for 1 generation.

† The enzymes encoded by these plasmids were inducible in *P. aeruginosa*.

‡ The enzymes encoded by these plasmids were not inducible in *P. aeruginosa*.

§ +O₂, well-aerated cultures; -O₂, cultures grown with limiting aeration.

Organization of the arcABC genes

CK and cOTC of *P. aeruginosa* have been purified; their subunit molecular masses are 37 kDa (Abdelal *et al.*, 1982) and 38 kDa (Legrain *et al.*, 1977), respectively. The ADI of *P. putida* has a subunit of 54 kDa (Shibatani *et al.*, 1978) whereas the ADI of *P. aeruginosa* has not yet been characterized. When extracts of induced *P. aeruginosa* cells carrying pME183 were subjected to SDS-polyacrylamide gel electrophoresis strong bands of about 38 and 50 kDa were visible; the 50 kDa band was missing from extracts of strain PTO6096/pME235 (ArcA⁻-LacZ⁺) (data not shown). We tentatively assign the 50 kDa peptide to the *arcA* gene. According to the enzyme subunit sizes, the *arcABC* structural genes are expected to occupy about 3.5 kb. The three genes were localized on a 3.8 kb segment between the Tn5-751 insertion of strain PAO6096 and the unique *Sma*I site (Fig. 1), which was used in the construction of pME190 (ArcA⁺B⁺C⁺) (Fig. 2). We conclude that the *arcABC* genes are contiguous.

The gene order was deduced from subcloning experiments performed in *E. coli*. The positions of *arcB* and *arcC* were defined by pME179, pME185, pME186, pME187, pME188 and pME191 (Fig. 2). The approximate location of *arcA* was determined by the analysis of pME100, pME195, pME197, pME235 and pME246 (Fig. 2). Fusion of *arcBC* and *arcA* fragments to strong vector promoters such as P_{Tac} (*E. coli* consensus promoter) or P_{Neo} (promoter of the Tn5 kanamycin/neomycin resistance gene) boosted the expression of the corresponding enzymes in *E. coli* (see pME179, pME188 and pME246; Fig. 2). From this type of experiment and from transcriptional *lacZ* (β -galactosidase) fusions in *arcA* (pME235, pME239) and *arcB* (pME244), the direction of transcription was deduced to be *arcABC* (Fig. 2). The 4.1 kb *lacZ* fragment which was used for the transcriptional fusions (Fig. 2) lacks the *lac* promoter and has only low intrinsic promoter activity in *E. coli*, corresponding to about 200 Miller units of β -galactosidase (Kroos & Kaiser, 1984). The same *lacZ* fragment gave much higher β -galactosidase levels, 4000–5000 Miller units, in *P. aeruginosa*, independent of the effects of external promoters (as in pKT240*lacZ*; Fig. 2). The β -galactosidase specified by the *arc-lacZ* fusion plasmids pME235 and pME244 was inducible by oxygen limitation in *P. aeruginosa* (Fig. 2). However, because of the accidental *Pseudomonas* promoter within the *lacZ* fragment, the β -galactosidase assays could not be used for a quantitative determination of *arc* enzyme induction.

There was no evidence for promoters between *arcA*, *arcB* and *arcC*. For instance, pME192 specified very low, non-inducible cOTC levels in *P. aeruginosa* and pME191 was devoid of *arcC* activity (Fig. 2).

Region upstream of arcABC

Part of the 1.6 kb region between the unique *Hind*III site and the presumptive start of the *arcA* gene (Fig. 2) was required for transcriptional initiation and regulation of the *arcABC* operon. The first evidence comes from pME100 (ArcA⁺B⁺C⁺), which carries the *arcABC* structural genes but not a 1.1 kb upstream region present in pME183 (Fig. 2). *P. aeruginosa* strains PAO6094, PTO6095 and PTO6096 harbouring pME100 expressed high levels of ADI [80 units (mg protein)⁻¹] and cOTC [1600 units (mg protein)⁻¹] under aerobic conditions. These levels were elevated only slightly (about twofold) by oxygen limitation. It seems likely that the region necessary for control by oxygen limitation is not intact in pME100. There is also a possibility that a 3.8 kb *Xho*I chromosomal fragment (not shown in Fig. 2), which was inserted accidentally in front of *arcABC* during cloning and which is unrelated to the *arc* operon, carries a strong promoter overriding the oxygen control of *arcABC* transcription.

Additional evidence for an *arc* regulatory region comes from the properties of strain PTO6091 (ArcA[±]B[±]C[±]). The Tn5-751 insertion (Fig. 1) in this non-inducible mutant had a *cis*-dominant effect on *arc* enzyme expression. This was shown by strain PTO6091 carrying pME244 (ArcA⁺ ArcB⁻-LacZ⁺ ArcC⁻; Fig. 2), in which the plasmid-encoded β -galactosidase (Fig. 2) and ADI were inducible by oxygen limitation, whereas the product of the chromosomal *arcB* gene, cOTC, was not. The specific activities [in units (mg protein)⁻¹] of ADI were 7 (good aeration) and 55 (limiting aeration), and those of cOTC were 14 (good aeration) and 10 (limiting aeration). Furthermore, pME197 (ArcA⁺B⁻C⁻; Fig. 2) did not complement in *trans* the mutant PTO6091 for anaerobic growth, whereas the *arcA* mutant PAO6094 was complemented by

pME197. We suggest that the Tn5-751 insertion of strain PTO6091 lies within the *arcABC* transcriptional unit.

DISCUSSION

ADI, cOTC and CK are coordinately induced (Mercenier *et al.*, 1980, 1982); their structural genes are highly cotransducible (Vander Wauven *et al.*, 1984). This indicates that the *arcABC* genes may be organized as an operon. In this study we present evidence that the *arcABC* genes are indeed contiguous and transcribed in the same direction, presumably as a unit. The 1.6 kb segment upstream of *arcA* appears to carry control regions which are necessary in *cis* for regulation by energy depletion and arginine. Additional functions required for anaerobic growth on arginine (e.g. the hypothetical *arcD* locus) may also be located there. The *P. aeruginosa arcD* mutants have wild-type levels of ADI, cOTC, and CK, and yet cannot grow anaerobically on arginine; *arcD* mutations are highly cotransducible with *arcB* (Vander Wauven *et al.*, 1984). Plasmid pME183 (ArcA⁺B⁺C⁺) restored anaerobic arginine catabolism in the *arcD* mutant PAO6138 (data not shown) and therefore appears to carry the *arcD* determinant, whose physiological function might be to provide anaerobic arginine transport.

Pseudomonas genes are generally weakly expressed in *E. coli* (Holloway, 1984; Minton & Clarke, 1985; Jeenes *et al.*, 1986) and the *arcABC* genes are no exception. Assuming a similar copy number for pME183 in *E. coli* and *P. aeruginosa*, the non-induced ADI and cOTC levels in *E. coli* were about 1% of those measured in *P. aeruginosa* (Table 2, Fig. 2). Fusion of the *arc* genes to good *E. coli* promoters strongly enhanced expression of the *arc* enzymes in *E. coli*. This suggests that the promoter(s) of *arcABC* are poorly recognized by the *E. coli* transcription machinery and supports the view that inefficient transcriptional initiation is the principal reason for the low expression of *Pseudomonas* genes in *E. coli* (Buckel & Zehelein, 1981; Minton & Clarke, 1985; Jeenes *et al.*, 1986).

We thank Marianne Gamper for communication of unpublished results, Patrizia Eppler-Kälin for technical assistance, David Jeenes for critically reading the manuscript and Antje Hitz for typing it. We are grateful to Victor Stalon and Thomas Leisinger for discussion and to E. Lanka and M. Bagdasarian for vector plasmids. This work was supported by a grant of the Swiss Federal Institute of Technology and, in part, by the Swiss National Foundation for Scientific Research (project 3.620-0.84).

REFERENCES

- ABDELAL, A. T., BIBB, W. F. & NAINAN, O. (1982). Carbamate kinase from *Pseudomonas aeruginosa*: purification, characterization, physiological role, and regulation. *Journal of Bacteriology* **151**, 1411–1419.
- BAGDASARIAN, M. M., AMANN, E., LURZ, R., RUECKERT, B. & BAGDASARIAN, M. (1983). Activity of the hybrid *trp-lac(tac)* promoter of *Escherichia coli* in *Pseudomonas putida*. Construction of broad-host-range, controlled-expression vectors. *Gene* **26**, 273–282.
- BORCK, K., BEGGS, J. D., BRAMMAR, W. J., HOPKINS, A. S. & MURRAY, N. E. (1976). The construction in vitro of transducing derivatives of phage lambda. *Molecular and General Genetics* **146**, 199–207.
- BUCKEL, P. & ZEHELEIN, E. (1981). Expression of *Pseudomonas fluorescens* D-galactose dehydrogenase in *E. coli*. *Gene* **16**, 149–159.
- DRETZEN, G., BELLARD, M., SASSONE-CORSI, P. & CHAMBON, P. (1981). A reliable method for the recovery of DNA fragments from agarose and acrylamide gels. *Analytical Biochemistry* **112**, 295–298.
- FRÜH, R., WATSON, J. M. & HAAS, D. (1983). Construction of recombination-deficient strains of *Pseudomonas aeruginosa*. *Molecular and General Genetics* **191**, 334–337.
- HAAS, D. (1983). Genetic aspects of biodegradation by pseudomonads. *Experientia* **39**, 1199–1213.
- HAAS, D., EVANS, R., MERCENIER, A., SIMON, J.-P. & STALON, V. (1979). Genetic and physiological characterization of *Pseudomonas aeruginosa* mutants affected in the catabolic ornithine carbamoyltransferase. *Journal of Bacteriology* **139**, 713–720.
- HAAS, D., MATSUMOTO, H., MORETTI, P., STALON, V. & MERCENIER, A. (1984a). Arginine degradation in *Pseudomonas aeruginosa* mutants blocked in two arginine catabolic pathways. *Molecular and General Genetics* **193**, 437–444.
- HAAS, D., CRYZ, S. J., JR, ITOH, Y., LEISINGER, T., LÜTHI, E., MERCENIER, A., REIMMANN, C., RELLA, M., SOLDATI, L., WATSON, J. M. & WRETLIND, B. (1984b). Some applications of transposon insertion mutagenesis in *Pseudomonas*. In *Génétique des Microorganismes Industriels*, pp. 91–111. Edited by H. Heslot. Paris: Société Française de Microbiologie.
- HOLLOWAY, B. W. (1984). Pseudomonads. In *Genetics*

- and *Breeding of Industrial Microorganisms*, pp. 63–92. Edited by C. Ball. Boca Raton, Florida: CRC Press.
- HOLMES, D. S. & QUIGLEY, M. (1981). A rapid boiling method for the preparation of bacterial plasmids. *Analytical Biochemistry* **114**, 193–197.
- ITOH, Y., WATSON, J. M., HAAS, D. & LEISINGER, T. (1984). Genetic and molecular characterization of the *Pseudomonas* plasmid pVS1. *Plasmid* **11**, 206–220.
- JEENES, D. J., SOLDATI, L., BAUR, H., WATSON, J. M., MERCENIER, A., REIMMANN, C., LEISINGER, T. & HAAS, D. (1986). Expression of biosynthetic genes from *Pseudomonas aeruginosa* and *Escherichia coli* in the heterologous host. *Molecular and General Genetics* **203**, 421–429.
- KROOS, L. & KAISER, D. (1984). Construction of Tn5 *lac*, a transposon that fuses *lacZ* expression to exogenous promoters, and its introduction into *Myxococcus xanthus*. *Proceedings of the National Academy of Sciences of the United States of America* **81**, 5816–5820.
- LEGRAIN, C., STALON, V., NOULLEZ, J.-P., MERCENIER, A., SIMON, J.-P., BROMAN, K. & WIAME, J.-M. (1977). Structure and function of ornithine carbamoyltransferases. *European Journal of Biochemistry* **80**, 401–409.
- MERCENIER, A., SIMON, J.-P., VANDER WAUVEN, C., HAAS, D. & STALON, V. (1980). Regulation of enzyme synthesis in the arginine deiminase pathway of *Pseudomonas aeruginosa*. *Journal of Bacteriology* **144**, 159–163.
- MERCENIER, A., STALON, V., SIMON, J.-P. & HAAS, D. (1982). Mapping of the arginine deiminase gene in *Pseudomonas aeruginosa*. *Journal of Bacteriology* **149**, 787–788.
- MILLER, J. H. (1972). *Experiments in Molecular Genetics*. Cold Spring Harbor: Cold Spring Harbor Laboratory.
- MINTON, N. P. & CLARKE, L. E. (1985). Identification of the promoter of the *Pseudomonas* gene coding for carboxypeptidase G2. *Journal of Molecular and Applied Genetics* **3**, 26–35.
- PALLERONI, N. J. (1984). Family I. *Pseudomonadaceae* Winslow, Broadhurst, Buchanan, Krunwiede, Rogers and Smith 1917, 555. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 141–219. Edited by N. R. Krieg. Baltimore: Williams & Wilkins.
- RELLA, M., MERCENIER, A. & HAAS, D. (1985). Transposon insertion mutagenesis of *Pseudomonas aeruginosa* with a Tn5 derivative: application to physical mapping of the *arc* gene cluster. *Gene* **33**, 293–303.
- SHIBATANI, T., KAKIMOTO, T. & CHIBATA, I. (1978). Subunit and amino acid composition of L-arginine deiminase of *Pseudomonas putida*. *FEBS Letters* **96**, 389–391.
- VANDER WAUVEN, C., PIÉRARD, A., KLEY-RAYMANN, M. & HAAS, D. (1984). *Pseudomonas aeruginosa* mutants affected in anaerobic growth on arginine: evidence for a four-gene cluster encoding the arginine deiminase pathway. *Journal of Bacteriology* **160**, 928–934.
- YANISCH-PERRON, C., VIEIRA, J. & MESSING, J. (1985). Improved M13 phage cloning vectors and host strains: nucleotide sequence of the M13mp18 and pUC19 vectors. *Gene* **33**, 103–119.