Isolation and characterization of *Pseudomonas putida* mutants affected in arginine, ornithine and citrulline catabolism: function of the arginine oxidase and arginine succinyltransferase pathways

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Pseudomonas putida mutants impaired in the utilization of arginine are affected in either the arginine succinyltransferase pathway, the arginine oxidase route, or both. However, mutants affected in one of the pathways still grow on arginine as sole carbon source. Analysis of the products excreted by both wild-type and mutant strains suggests that arginine is mainly channelled by the oxidase route. Proline non-utilizing mutants are also affected in ornithine utilization, confirming the role of proline as an intermediate in ornithine catabolism. Mutants affected in ornithine cyclodeaminase activity still grow on proline and become unable to use ornithine. Both proline non-utilizing mutants and ornithine-cyclodeaminase-minus mutants are unable to use citrulline. These results, together with induction of ornithine cyclodeaminase when wild-type *P. putida* is grown on citrulline, indicate that utilization of citrulline as a carbon source proceeds via proline with ornithine as an intermediate. Thus in *P. putida*, the aerobic catabolism of arginine on the one hand and citrulline and ornithine on the other proceed by quite different metabolic segments.

Introduction

Four arginine catabolic pathways are known in *Pseudomonas putida* (Fig. 1). The arginine deiminase pathway allows the use of arginine as a cellular ATP source under conditions of energy depletion (Stalon *et al.*, 1972; Stalon & Mercenier, 1984). The arginine decarboxylase pathway serves mainly for the production of polyamines under conditions of arginine abundance (Stalon & Mercenier, 1984). Two other pathways, the oxidase route (Miller & Rodwell, 1971) and the arginine succinyltransferase pathway (Stalon *et al.*, 1987) seem to be involved in the aerobic carbon catabolism of arginine. The fractional flow of intermediates along either of these arginine catabolic pathways is unknown. Characterization of structural or regulatory mutations affecting each pathway would provide a better insight into the nature and significance of the multiple routes. With the aim of assessing the physiological role of the arginine oxidase and arginine succinyltransferase routes, we have isolated mutants blocked in both pathways. We have also investigated the metabolism of ornithine in *P. putida*. Ornithine catabolism in *P. aeruginosa* requires as the first step succinylation of the α -nitrogen atom of ornithine (Vander Wauven *et al.*, 1988). From succinylornithine,

catabolism of ornithine uses, in this strain, metabolic segments common to the arginine succinyltransferase pathway (Jann *et al.*, 1986; Vander Wauven *et al.*, 1988). Succinylation of ornithine is common among *Pseudomonas* strains as well as in *Aeromonas* and the enterobacterium *Klebsiella aerogenes*, all of which are able to use ornithine as sole carbon source (Stalon *et al.*, 1987). *P. putida* seems to be devoid of ornithine succinyltransferase activity (Stalon *et al.*, 1987). We found, however, that *P. putida* converts ornithine to proline. The participation of an ornithine cyclodeaminase in the catabolism of ornithine has now been ascertained by the isolation of mutants affected in this activity and in proline utilization.

Methods

Bacterial strains and growth media. All mutants are derived from *P. putida* IRC204 (Stalon *et al.*, 1967). Strains were grown routinely at 30 °C in nitrogen-free minimal medium 154 (Stalon *et al.*, 1967). Substrates used as a carbon source or nitrogen source were added after separate sterilization. When used as a carbon source the final concentration of substrates was 20 mM. When used as a nitrogen source, substrates were usually included at a final concentration of 10 mM.

Fig. 1. Catabolism of arginine, citrulline and ornithine by *P. putida*. 1, Arginine oxidase (EC 1.4.3.-); 2, 2-oxoarginine decarboxylase (EC 4.1.1.-); 3, 4-guanidinobutyraldehyde oxidoreductase (EC 1.1.1.-); 4, guanidinobutyrate (EC 3.5.3.-); 5, arginine decarboxylase (EC 4.1.1.19); 6, agmatine deiminase (EC 3.5.3.12); 7, *N*-carbamoylputrescine hydrolase (EC 3.5.3.-); 8, putrescine transaminase (EC 2.6.1.-); 9, 4-aminobutyraldehyde dehydrogenase (EC 1.2.1.19); 10, 4-aminobutyrate transaminase (EC 2.6.1.19); 11, succinate semialdehyde dehydrogenase (EC 1.2.1.-); 12, arginine succinyltransferase (EC 2.3.1.-); 13, *N*²-succinylarginine dihydrolase (EC 3.5.3.-); 14, *N*²-succinylornithine aminotransferase (EC 2.6.1.-); 15, *N*²-succinylglutamate semialdehyde dehydrogenase (EC 1.2.1.-); 16, *N*²-succinylglutamate desuccinylase (EC 3.5.1.-); 17, arginine deiminase (EC 3.5.3.6); 18, ornithine carbamoyltransferase (EC 2.1.3.3); 19, carbamate kinase (EC 2.7.2.21); 20, ornithine cyclodeaminase (EC 4.1.1.17); 21, proline dehydrogenase (EC 1.5.99.8); 22, 1-pyrroline-5-carboxylate dehydrogenase (EC 1.5.1.12); 23, glutamate dehydrogenase (EC 1.4.1.3); 24, oxoglutarate synthase (EC 1.2.7.3); 25, succinate-CoA ligase (EC 6.2.1.5); 26, ornithine decarboxylase (EC 4.1.1.17).

Strains were tested for their ability to grow at the expense of arginine, citrulline, ornithine and potential intermediates in their catabolism. The tests were done in liquid media where substrate consumption and product excretion could be measured. To prepare inocula for substrate utilization studies, 3 ml of rich medium was inoculated from a slant and incubated overnight. Cells were washed twice with NaCl (0.9%, w/v) and then used to inoculate 3 ml of standard salt medium 154 at 10^7 cells ml^{-1} . Test-tubes were shaken and bacterial growth was followed by eye. For enzyme activity determination, cultures were grown in 100 ml batches in 1 litre flasks on a rotary shaker. Cells were harvested in the mid-exponential phase of growth, washed twice with NaCl (0.9%) and stored at -20°C . Bacterial growth was followed by measuring the optical density at 660 nm with a Beckman photometer.

Isolation of mutants. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) mutagenesis was performed as in Stalon *et al.* (1972). After three enrichment cycles using penicillin counterselection, suitable dilutions were plated on supplemented medium and mutants detected by replica-plating of the colonies. Mutants IRC204-7, IRC204-8 and IRC204-15 were selected for their inability to grow on guanidinobutyrate. Strain IRC204-28 was selected as a proline non-utilizing mutant, strain IRC204-202 as an ornithine non-utilizing mutant and strain IRC204-102 for its poor growth on arginine.

Enzyme assays. Unless otherwise stated, assays were performed on cell extracts prepared by sonication of cells harvested in the exponential growth phase (Stalon *et al.*, 1967) suspended in 50 mM-potassium phosphate, pH 7.5, containing 3 mM- β -mercaptoethanol. Cell debris was removed by centrifugation at 20000 *g* for 20 min. Extracts were kept at 4°C . Protein concentrations were determined by the Lowry method. All enzymes were assayed at 30°C . One unit of enzyme is defined as the amount of enzyme catalysing the formation of 1 μmol of product h^{-1} . Specific activities are units (mg protein) $^{-1}$. The various enzyme activities were assayed as follows. Enzymes involved in the arginine succinyltransferase pathway, namely arginine succinyltransferase (EC 2.1.3.-), *N*²-succinylarginine dihydrolase (EC 3.5.3.-), *N*²-succinylornithine aminotransferase (EC 2.6.1.-) and *N*-succinylglutamate desuccinylase (EC 3.5.1.-) according to Vander Wauven & Stalon (1985). Guanidinobutyrase (EC 3.5.3.7) was assayed under the conditions given by Miller & Rodwell (1971) except that 4 mM- MnCl_2 was used in the assay; succinic semialdehyde dehydrogenase (EC 1.2.1.16) was determined from the increase in absorbance at 340 nm corresponding to NADP reduction (Jacoby, 1962). 4-Aminobutyrate transaminase (EC 2.6.1.19) was assayed according to Jacoby (1962) by coupling with succinic semialdehyde dehydrogenase. Ornithine cyclodeaminase (EC 4.1.1.17) and ornithine transaminase (EC 2.6.1.13) were assayed according to Stalon *et al.* (1987).

Analytical methods. The presence of urea in the culture supernatant was detected either by the colorimetric method of Archibald (1944) or of Prescott & Jones (1969); guanidino- derivatives were determined according to Miklus & Stein (1973). The presence of amino- and guanidino- derivatives was also identified by high voltage electrophoresis (Vander Wauven & Stalon, 1985).

Chemicals. Amino acids, putrescine, 4-aminobutyrate, guanidinobutyrate, succinic semialdehyde, succinyl-CoA and aminooxyacetic acid were obtained from Sigma. Agmatine was purchased from Aldrich. *N*-Carbamoylputrescine was prepared according to Mercenier *et al.* (1980) and 2-oxoarginine was obtained as described in Stalon & Mercenier (1984). Succinylarginine, succinylornithine and succinylglutamate preparations were according to Vander Wauven & Stalon (1985).

Results and Discussion

Characterization of mutants

P. putida mutants unable to use guanidinobutyrate as sole carbon and nitrogen source were obtained after mutagenesis with NTG followed by three penicillin enrichment cycles. Selection medium contained 20 mM-guanidinobutyrate as carbon and nitrogen source; enrichment medium was 20 mM-succinate plus 10 mM-ammonium salt. Among the mutants obtained 84% (65 mutants), represented by strain IRC204-7 were still able to use 4-aminobutyrate as carbon and nitrogen source and grow at a reduced rate on arginine (Table 1); 8% (six mutants), represented by mutant IRC204-8 were unable to use guanidinobutyrate and arginine as sole carbon source, although they could use arginine as a nitrogen source; 8% (six mutants), represented by strain IRC204-15 had lost the ability to use both guanidinobutyrate and 4-aminobutyrate – they were blocked in agmatine, carbamoylputrescine and putrescine utilization and also grew at a reduced rate on arginine (Table 1).

Mutant IRC204-102 was selected for its poor growth in the presence of arginine; the selection medium contained citrate and arginine, the enrichment medium contained putrescine.

Proline non-utilizing mutants were selected on 20 mM-proline medium and enriched on glutamate medium. Mutant IRC204-28 is representative of this class of mutants; it is blocked in citrulline and ornithine utilization but grew well on arginine, agmatine or 2-oxoarginine (Table 1). Mutants blocked between ornithine and proline were selected on ornithine medium and enriched on proline medium. The growth characterization of these mutant strains are summarized in Table 1.

Enzyme activities in the arginine oxidase and arginine succinyltransferase pathways

Enzyme activities of guanidinobutyrase, 4-aminobutyrate transaminase and succinic semialdehyde dehydrogenase in the arginine oxidase pathway as well as enzymes involved in the arginine succinyltransferase pathway were measured in wild-type and mutant strains after growth on various different media. The enzymes of the succinyltransferase pathway were induced in arginine medium and the specific activities varied considerably in the presence of different carbon sources (Table 2). There were no significant differences in enzyme synthesis between strains IRC204, IRC204-7 and IRC204-15 (data not shown). Mutant IRC204-102 is impaired in the succinylarginine dihydrolase activity whereas mutant

Table 1. *Growth of wild-type and mutant strains*

Growth was followed in liquid medium 154 in the presence of 10 mM-ammonium salt and the listed substrates tested at 20 mM. Growth is indicated as +++ for good growth decreasing to + for poor growth and - indicating no growth. Tubes were incubated for 1 to 2 d at 30 °C. NT, Not tested.

Growth substrate	IRC204	IRC204-7	IRC204-8	IRC204-15	IRC204-102	IRC204-28	IRC204-202
Glutamate	+++	+++	+++	+++	+++	+++	+++
L-Arginine	+++	+	-	+	+	++	++
D-Arginine	++	+	-	+	+	++	++
Oxoarginine*	+++	-	-	-	++	NT	NT
Agmatine	+++	+++	+++	-	+++	+++	+++
Carbamylputrescine	+++	+++	+++	-	+++	+++	+++
Putrescine	+++	+++	+++	-	+++	+++	+++
Guanidinobutyrate	+++	-	-	-	+++	+++	+++
4-Aminobutyrate	+++	+++	+++	-	+++	+++	+++
Succinic semialdehyde	++	++	++	++	++	++	++
Citrulline	+++	+++	+++	+++	+++	-	-
Ornithine	++	++	++	++	++	-	-
Proline	+++	+++	+++	+++	+++	-	+++

* Due to some contamination of oxoarginine by arginine, positive growth was indicated by disappearance of more than 90% of the guanidino- moiety with concomitant appearance of the urea formed. Negative growth was assumed if, after 3 d incubation, less than 15% of the guanidino- compound had been consumed.

Table 2. *Enzyme synthesis in the arginine succinyltransferase pathway in P. putida IRC204*

Cells were grown in minimal medium with the growth substrates listed. Values marked *a* are taken from Stalon *et al.* (1987).

Strain	Growth substrate(s)	Enzyme specific activity [units (mg protein) ⁻¹]			
		Arginine succinyl-transferase*	Succinyl-arginine dihydrolase†	Succinyl-ornithine transaminase*	Succinyl-glutamate desuccinylase†
IRC204	Citrate + NH ₄ ⁺	0.2 ^a	0.9	5.0 ^a	0.7
	Guanidinobutyrate	0.7	3.0	3.0	0.6
	Arginine	12.6 ^a	14.5	20.0 ^a	7.7
	Citrulline	2.2	2.3	5.6 ^a	1.1
	Ornithine	0.8 ^a	1.3	2.7 ^a	1.2
	Arginine/glutamate	6.3	8.5	10.1	6.5
IRC204-102	Arginine	11.4	0.05	18.0	5.5
IRC204-8	Arginine/glutamate	0.2	0.2	3.0	0.2

* Mean values from at least duplicate independent experiments with less than 10% variation in activity.

† Values from a single experiment.

IRC204-8 has a severe reduction of all enzyme activities (Table 2).

Guanidinobutyrase, 4-aminobutyrate transaminase and succinate semialdehyde dehydrogenase were highly induced by growing wild-type cells on either 2-oxoarginine or guanidinobutyrate (Table 3). The levels of these enzymes were, however, lower on arginine medium.

Strain IRC204-102, affected in succinylarginine dihydro-lase activity, had levels of all three enzymes similar to the wild-type strain, on arginine as well as on guanidinobutyrate medium (data not shown).

Strain IRC204-7 was impaired in guanidinobutyrase activity (Table 3). This mutant had the same level of transaminase and dehydrogenase activities on 4-amino-

Table 3. *Enzymes synthesis in the arginine oxidase pathway in P. putida IRC204*

Cells were grown in minimal media with the growth substrates listed. Enzyme specific activities are mean values of at least two independent determinations with less than 10% variation in activity. NT, Not tested.

Strain	Growth substrate(s)	Enzyme specific activity [units (mg protein) ⁻¹]		
		Guanidino- butyrase	4-Amino- butyrate transaminase	Succinic semialdehyde dehydrogenase
IRC204	Citrate/NH ₄ ⁺	0.2	1.9	11.5
	Guanidinobutyrate	38.0	30.0	70.0
	Oxoarginine	40.0	NT	NT
	4-Aminobutyrate	<0.2	33.0	67.0
	Succinate semialdehyde/NH ₄ ⁺	<0.2	2.6	28.0
	Agmatine	0.5	20.0	89.0
	Putrescine	0.2	18.5	33.0
	Arginine	11.5	5.1	38.0
	Citrate/arginine	5.7	3.3	18.0
	Citrate/guanidinobutyrate	14.0	14.0	50.0
IRC204-7	Citrate/arginine	<0.2	0.7	13.4
IRC204-8	Citrate/arginine	<0.2	0.5	8.7
IRC204-15	Citrate/arginine	<0.2	0.1	158.0

butyrate medium as the wild-type strain (data not shown). When this mutant was grown on arginine, as no 4-aminobutyrate can be formed *in vivo* from arginine, a severe reduction in transaminase and dehydrogenase syntheses was observed (Table 3). Mutant IRC204-15 is affected in both guanidinobutyrase and transaminase activities. The fact that this strain was unable to use agmatine, carbamoylputrescine or putrescine, besides guanidinobutyrate and 4-aminobutyrate, indicates that the agmatine catabolic pathway converges to 4-aminobutyrate. In the wild-type strain, the inducing capacity of agmatine, putrescine, arginine or guanidinobutyrate for succinate semialdehyde dehydrogenase and 4-aminobutyrate transaminase may be related to the ability of these various growth substrates to be metabolized to 4-aminobutyrate (Table 3). Nevertheless, mutant IRC204-15, affected in both guanidinobutyrase and transaminase activities had a depressed dehydrogenase activity on arginine medium. This suggests that besides mutations in the structural genes, the general regulatory mechanism could be affected as well. The complete failure of mutant IRC204-8 to grow on arginine could be explained by the severe deficiency in both the arginine oxidase and the arginine succinyltransferase pathways (Tables 2 and 3). Multiple pathways for the catabolism of one amino acid may also lead to the isolation of mutants with lesions in the general control mechanism or in the transport system. Strain IRC204-8 could still use arginine as a nitrogen source. The non-induced levels of the enzymes in the two pathways, as well as that of arginine

deiminase, were probably sufficient to account for growth with arginine as a nitrogen source. Growth was, however, distinctly slower with a generation time of ≥ 400 min on citrate plus arginine as compared to 60 min for the wild-type strain in the same conditions. This poor growth might reflect an impairment in the ability of the mutant to take up arginine so that the amount of arginine entering the cell was too low to allow induction of the catabolic enzymes.

Growth characteristics of wild-type and mutant strains

P. putida IRC204 grew on arginine as sole carbon and nitrogen source with a doubling time of 60 min. Arginine utilization was characterized by an abundant excretion of urea. However, *P. putida* was able to use urea as sole nitrogen source; therefore quantitative analysis of excreted urea was followed in the presence of 10 mM ammonium salt, thus imposing repression of urease (Tricot *et al.*, 1990). Consequently, under these conditions the amount of urea excreted by cells of *P. putida* growing on arginine as sole carbon source is a rough estimate of the functioning of the arginine oxidase pathway. Careful analysis of arginine consumed and urea produced revealed that 60% of the guanidino group of arginine was found in the form of excreted urea. Among other products found in the spent culture and analysed by high voltage electrophoresis, there was some citrulline and ornithine produced by the non-induced levels of enzymes of the arginine deiminase route.

Resting cells incubated with 10 mM-arginine and 10 mM-aminooxyacetic acid, an inhibitor of pyridoxal phosphate dependent enzymes (Wilson & Holden, 1969), accumulated, besides urea, ornithine, citrulline, succinylornithine and 4-aminobutyrate, indicating that the arginine catabolic pathways are blocked at the level of succinyltransferase and 4-aminobutyrate transaminase. Another conclusion is that L-arginine is directly converted into 2-oxoarginine without D-arginine as an intermediate as found in *P. aeruginosa* (Jann *et al.*, 1987). D-Arginine is also a good carbon source for *P. putida*. Resting cells grown on D-arginine and incubated with 10 mM-D-arginine + 10 mM-aminooxyacetic acid did not accumulate urea, 4-aminobutyrate or succinylornithine. This is expected if a pyridoxal-phosphate-dependent racemase is involved in the interconversion of the enantiomers and D-arginine utilization involves L-arginine as an intermediate.

Mutant IRC204-7 grew on arginine with a doubling time of 180 min whereas mutant IRC204-102 had a doubling time of 300 min. This suggests that the contribution of the arginine succinyltransferase pathway to arginine utilization is more important than the oxidase route. However, this is in conflict with the results obtained on product excretion. Mutant IRC204-7 growing on arginine produced no urea but 2-oxoarginine, guanidinobutyrate and an unidentified guanidino- compound, probably guanidinobutyraldehyde. In contrast, when arginine (10 mM) was entirely consumed by cells of strain IRC204-102, most of the guanidino- moiety was recovered as excreted urea (55–60%) and 35–40% was recovered as succinylarginine. Consequently, the lower growth rate on arginine of strain IRC204-102 may be explained by consumption of succinyl-CoA which cannot be regenerated because of a defect in succinyl-arginine dihydrolase. It was previously shown for *P. aeruginosa* that addition of arginine to glutamate-growing cells of a mutant blocked in succinyl dihydrolase arrests growth (Vander Wauven *et al.*, 1988).

Ornithine catabolism

Ornithine is itself an important point of metabolic divergence since it is susceptible to several modes of enzymic conversion. Ornithine utilization may occur via arginine formation by the final steps of arginine synthesis. However, ornithine catabolism via arginine synthesis is unlikely since mutants blocked in either the arginine oxidase or the arginine succinyltransferase pathway still grow on ornithine as well as the wild-type strains does (Table 1). Transamination of ornithine to glutamate semialdehyde has been observed in many bacteria (see Cunin *et al.*, 1986). An ornithine transamin-

ase activity induced by arginine has been observed (Stalon *et al.*, 1987). This activity was not synthesized by cells growing on ornithine. As in *P. aeruginosa* and other *Pseudomonas* strains having the arginine succinyltransferase pathway, this activity in *P. putida* was probably authentic succinylornithine transaminase (Stalon *et al.*, 1987; Vander Wauven *et al.*, 1988).

Although ornithine decarboxylase was detected in cell extracts, a pathway involving this enzyme appears an unlikely route for ornithine utilization as a carbon source since strain IRC204-15, a putrescine non-utilizing mutant, still grew on ornithine as a carbon source. Ornithine decarboxylase, which produces putrescine, is involved mainly in polyamine synthesis (see Cunin *et al.*, 1986).

Cells grown on ornithine contained an induced level of ornithine cyclodeaminase activity (Table 4). The obligatory involvement of ornithine cyclodeaminase and proline in the catabolism of ornithine is indicated by the fact that mutant IRC204-202 is devoid of cyclodeaminase activity and does not use ornithine as a carbon source, whereas proline non-utilizing mutants represented by strain IRC204-28 are also unable to use ornithine (Table 4).

Citrulline utilization and ornithine cyclodeaminase synthesis

Citrulline is a good carbon source for *P. putida* (doubling time 60 min) as compared to ornithine (doubling time 90 min). The obligatory involvement of ornithine and proline in citrulline utilization is indicated by the observations that (i) ornithine cyclodeaminase was induced by growing cells on citrulline, and (ii) proline non-utilizing mutants, represented by strain IRC204-28 as well as strain IRC204-202 lacking ornithine cyclodeaminase activity, failed to grow on citrulline. Maximum induction of cyclodeaminase activity occurred with citrulline as sole carbon and nitrogen source (Table 4). The lower level of cyclodeaminase found in ornithine-grown cells might suggest that induction of the enzyme by ornithine was poor so that insufficient enzyme to support good growth was formed. Addition of proline to cells growing on ornithine resulted in a higher growth rate (doubling time 55 min) and synthesis of ornithine cyclodeaminase. In Gram-negative bacteria proline regulates the osmotic equilibrium and may alter the permeability of the cytoplasmic membrane allowing solutes to permeate the cells more easily (Csonka, 1989). The stimulation of cyclodeaminase synthesis in arginine-grown cells suggests that arginine might induce cyclodeaminase activity only by virtue of its conversion to citrulline or ornithine mediated by the arginine deimin-

Table 4. Regulation of ornithine cyclodeaminase synthesis in *P. putida* IRC204

Cells were grown in minimal medium with the growth substrate(s) listed. Except for the two values marked with an asterisk (*), which are from a single experiment, the values shown are means from two independent experiments (enzyme assays done in duplicate); variation was less than 10%.

Strain	Growth substrate(s)	Enzyme specific activity [units (mg protein) ⁻¹]
IRC204	Citrate + NH ₄ ⁺	<0.05
	Citrulline	7.2
	Ornithine	2.0
	Arginine	4.1
	Citrulline + ornithine	9.9
	Citrulline + proline	6.2
	Ornithine + proline	9.0
	Proline	<0.05
	Citrulline + citrate	5.8*
	Citrulline + NH ₄ ⁺	6.6
	Citrulline + glutamate	6.0
	Citrulline + citrate + NH ₄ ⁺	5.8*
	Citrulline + glutamine	3.9
	Citrulline + citrate + glutamine	3.0
IRC204-28	Citrulline + glutamate	5.5
IRC204-202	Citrulline + glutamate	<0.1

ase pathway. Some citrulline was always found to be excreted from cells growing on arginine under aerobiosis whereas abundant ornithine excretion was observed in cells grown on arginine during an anaerobic shock (Stalon & Mercenier, 1984). The activity responsible for citrulline/ornithine interconversion is probably the catabolic ornithine carbamoyltransferase (OTCase). Consequently, catabolic OTCase fulfills two functions: one is the utilization of arginine for synthesis of ATP in anaerobiosis (Stalon & Mercenier, 1984); the second concerns citrulline utilization in aerobiosis. Mutants affected in the structural gene encoding catabolic OTCase will be of interest to determine the physiological role of this enzyme as well as the distribution of the arginine flux along this pathway in *P. putida*.

Conclusions

The results of this study show that both the oxidase and the arginine succinyltransferase pathways are essential for sustaining full wild-type growth rates on arginine used as sole carbon source. The loss of one pathway by deficiency in a structural gene causes bradytrophic growth. The neotype strain *P. putida* ATCC 12633, as well as strain ATCC 25571 (isolated by Miller & Rodwell, 1971), in which the arginine oxidase route was identified, also have both pathways (unpublished observations). Most *Pseudomonas* strains use only the arginine succinyltransferase route for aerobic argin-

ine utilization (Stalon *et al.*, 1987), although the pathway leading to guanidinobutyrate is cryptic in some strains (Jann *et al.*, 1988; Yorifuji *et al.*, 1983).

Another characteristic typical of *P. putida* when compared to other *Pseudomonas* strains is its ability to use citrulline as sole carbon source. Citrulline utilization involves ornithine and proline as intermediates whereas most *Pseudomonas* strains use the ornithine succinyltransferase pathway. *P. putida* converts ornithine to proline by means of ornithine cyclodeaminase, an activity which is also found in *Clostridium sporogenes* (Costilow & Laycock, 1969, 1971), *Treponema denticola* (Leschine & Canale-Parola, 1980), *Agrobacterium tumefaciens* (Dessaux *et al.*, 1986) and *P. cepacia* (Stalon *et al.*, 1987).

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