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L-Lysine Catabolism Is Controlled by L-Arginine and ArgR in *Pseudomonas aeruginosa* PAO1▽

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In comparison to other pseudomonads, *Pseudomonas aeruginosa* grows poorly in L-lysine as a sole source of nutrient. In this study, the *ldcA* gene (*l*ysine *d*e*c*arboxylase *A*; PA1818), previously identified as a member of the ArgR regulon of L-arginine metabolism, was found essential for L-lysine catabolism in this organism. LdcA was purified to homogeneity from a recombinant strain of *Escherichia coli*, and the results of enzyme characterization revealed that this pyridoxal-5-phosphate-dependent decarboxylase takes L-lysine, but not L-arginine, as a substrate. At an optimal pH of 8.5, cooperative substrate activation by L-lysine was depicted from kinetics studies, with calculated K_m and V_{max} values of 0.73 mM and 2.2 μmole/mg/min, respectively. Contrarily, the *ldcA* promoter was induced by exogenous L-arginine but not by L-lysine in the wild-type strain PAO1, and the binding of ArgR to this promoter region was demonstrated by electromobility shift assays. This peculiar arginine control on lysine utilization was also noted from uptake experiments in which incorporation of radioactively labeled L-lysine was enhanced in cells grown in the presence of L-arginine but not L-lysine. Rapid growth on L-lysine was detected in a mutant devoid of the main arginine catabolic pathway and with a higher basal level of the intracellular L-arginine pool and hence elevated ArgR-responsive regulons, including *ldcA*. Growth on L-lysine as a nitrogen source can also be enhanced when the *aruH* gene encoding an arginine/lysine:pyruvate transaminase was expressed constitutively from plasmids; however, no growth of the *ldcA* mutant on L-lysine suggests a minor role of this transaminase in L-lysine catabolism. In summary, this study reveals a tight connection of lysine catabolism to the arginine regulatory network, and the lack of lysine-responsive control on lysine uptake and decarboxylation provides an explanation of L-lysine as a poor nutrient for *P. aeruginosa*.

Decarboxylation of amino acids, including lysine, arginine, and glutamate, is important for bacterial survival under low pH (2, 7, 19). Lysine is abundant in the rhizosphere where fluorescent *Pseudomonas* preferentially resides, and serves as a nitrogen and carbon source to these organisms (28). In microbes, lysine catabolism can be initiated either through monooxygenase, decarboxylase, or transaminase activities. The monooxygenase pathway has been considered the major route for L-lysine utilization in *Pseudomonas putida*, and *davBATD* encoding enzymes for the first four steps of the pathway have been characterized (25, 26). In contrast, *Pseudomonas aeruginosa* cannot use exogenous L-lysine efficiently for growth (5, 24). It has been reported that enzymatic activities for the first two steps of the monooxygenase pathway are not detectable in *P. aeruginosa*, and no *davBA* orthologs can be identified from this organism (24, 25).

Mutants of *P. aeruginosa* with improved growth on L-lysine and a high level of lysine decarboxylase activity can be isolated by repeated subcultures in L-lysine (5). This suggests that in *P. aeruginosa*, L-lysine utilization might be mediated by the lysine decarboxylase pathway with cadaverine and 5-aminovalerate as intermediates (Fig. 1). Alternatively, conversion of L-lysine into 5-aminovalerate may also be accomplished by a coupled reaction catalyzed by AruH and AruI. The AruH and AruI enzymes were reported as arginine:pyruvate transaminase and 2-ketoarginine decarboxylase, respectively (36). Interestingly, transamination by AruH using L-lysine as an amino group donor can also be detected *in vitro* (35). The reaction product α-keto-ε-aminohexanonate can potentially be decarboxylated into 5-aminovalerate by AruI, providing an alternative route for lysine degradation.

In this study, we showed that the lysine decarboxylase pathway is the main route for lysine utilization under arginine control. Expression of the *ldcAB* operon encoding L-lysine decarboxylase and a putative lysine/cadaverine antiporter was analyzed regarding its response to L-lysine, L-arginine, and the arginine-responsive regulator ArgR. Enzyme characterization was performed to verify the function of LdcA as L-lysine decarboxylase. Arginine control on lysine incorporation was also investigated by genetic studies and uptake experiments. The peculiar role of ArgR controlling arginine and lysine uptake and catabolism provides the explanation for poor growth in lysine, and it implies a higher level of complexity in metabolic networks of pseudomonads.

MATERIALS AND METHODS

Bacterial strains, plasmids, media, and growth conditions. Bacterial strains and plasmids used in this study are listed in Table 1. *Escherichia coli* and *P. aeruginosa* strains were grown in Luria-Bertani (LB) medium supplemented with antibiotics when indicated at conventional concentrations (36). The minimal medium P (MMP) containing the indicated carbon and nitrogen sources at 20 mM and 5 mM, respectively, was used for the growth of *P. aeruginosa* (8).

Construction of *ldcA*::*lacZ* fusion. The upstream regulatory region of the *ldcAB* operon covering 635 bp upstream of the start codon and the first 24 bp of

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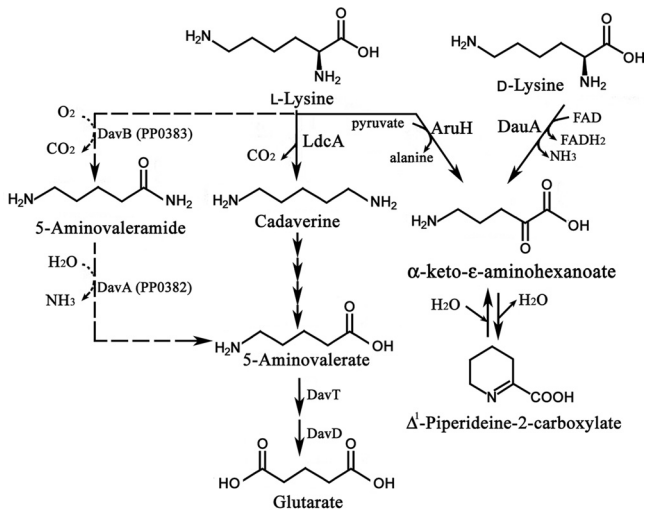

FIG. 1. Lysine catabolic pathways. L-lysine decarboxylase pathway is shown at center. Broken arrows represent lysine monooxygenase pathway from *P. putida* which is not present in *P. aeruginosa*.

the structural gene was PCR amplified from the genomic DNA of *P. aeruginosa* PAO1 using the following primers: 5′-TCACTCTGGGCGCAAGCTTAGGCG CCGGTCGGC-3′ and 5′-GGGAAATTTGAGGTCTTT-3′. PCR product was cloned into pQF50 and confirmed by DNA sequencing.

Construction of mutant strains. For tetracycline-resistant mutants, DNA fragments covering the genes of interest were PCR amplified from PAO1 genomic DNA using the following primers: for *ldcAB* mutants, 5′-ATGTATAAAGACC TCAAATTTCCCGTCCTC-3′ and 5′-TCAGTCATTGGCTTTGAGCGTCGG CACTCC-3′; and for *aruHI* mutants, 5′-GTCTAAGCTTGACTGGCCTGGCG CGCGTCG-3′ and 5′-CGCAAGCTTCGGGCAGTCCGGCGTGACCCT-3′. PCR products were cloned into a conjugation vector, pRTP1 (32), and the tetracycline resistance cassette was introduced by using the EZ-Tn*5* <TET-1> insertion system (Epicentre). For gentamicin deletion mutants, two flanking regions of a targeted gene were amplified by PCR with the following primers: 5′-GAGGGATCACTCGGGTGCATACTTCTTCTACG-3′, 5′-GAGGAATTC AGCGCT TCCAGGTCGTTGTAG-3′, 5′-GAGGAATTCGAGCGCTACGTC GAACAGGACATGA-3′ and 5′-GAGAAGCTTCAATCCGAGCAGGTTGC TCATGGTC-3′. The PCR products were cloned into pRTP1. A cassette carrying the gentamicin resistance gene from pGMΩ1 was inserted into the conjunction of the two DNA fragments (29). For gene replacement, *E. coli* SM10 was served as the donors in biparental mating with PAO1-Sm^r (6). The desired knockout mutants were selected on LB plates containing streptomycin and either gentamicin or tetracycline, and the mutation was confirmed by PCR.

Constitutive expression from pUCP18. Full-length *ldcA* (*PA1818*) and *aruIH* (*PA4976* and *PA4977*) genes were successfully PCR amplified and subcloned into pUCP18 to construct pHC5306 and pHC5307 using the following primers: PA1818F, 5′-TGAAGATCTGAGGAGTCAACAATGTATAAAGACCTC-3′; PA1818R, 5′-CCCAAGCTTTCATTCCTTTATGCATTCAACGGT-3′; PA4976F 5′-CACGGATCCGCTTGTGGGAATGGGAGCAAGAGC-3′; and PA4976R, 5′-CACAAGCTTCATCGTGGTTTCCGAATCG TGGTGAC-3′. These plasmids can reach 10 to 25 copy numbers per cell with constitutive expression in *P. aeruginosa* (30).

Measurements of β-galactosidase activity. The cells were grown in the minimal medium P containing the carbon and nitrogen sources as indicated. Cells in

TABLE 1. Strains and plasmids

Strain or plasmid	Genotype or description[a]	Source or reference
E. coli strains		
DH5α	F$^-$ ϕ80d*lac* ΔM15 Δ(*lacZYA-argF*)*U169 deoR recA1 endA1 hsdR17* (r_K^- m_K^-) *supE44* λ$^-$ *thi-1 gyrA96 relA*	Bethesda Research Laboratories
TOP10	F$^-$ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) ϕ80*lacZ*ΔM15 Δ*lacX74 recA1 araD139* Δ(*ara-leu*)*7697 galU galK rpsL* (Sm^r) *endA1 nupG*	Invitrogen
SM10	*thi-1 thr leu tonA lacY supE recA*::RP4-2-Tc::Mu (Km^r)	31
P. aeruginosa strains		
PAO1	Wild type	8
PAO1-Sm^r	Spontaneous Sm^r mutant strain of PAO1	11
PAO501	*argR*::Gm^r	23
PAO1214	*aotJ*::Tn*5-751* insertion mutant of PAO1	23
PAO5715	Δ*aruCFGDBE*::Gm^r	This study
PAO5716	*ldcA*::Tc^r	This study
PAO5717	*ldcB*::Tc^r	This study
PAO5602	*aruH*::Tc^r Δ*aruF*	36
PAO5718	Δ*aruCFGDBE*::Gm^r *ldcA*::Tc^r	This study
PAO5720	*aruH*::Tc^r	This study
PAO5721	*aruI*::Tc^r	This study
PAO5802	*dauA*::Tc^r	8, 14
Plasmids		
pRTP1	Amp^r Sm^s conjugation vector	32
pRTP2	pRTP1 derivative; EcoRI site deleted	13
pGMΩ1	*bla gen*; gentamicin resistance gene cassette with omega loop on both ends	29
pUCP18	*Escherichia-Pseudomonas* shuttle vector	30
pHC5306	pUCP18 derivative carrying *ldcA* gene	This study
pHC5307	pUCP18 derivative carrying *aruH* gene	This study
pBAD-His6	Modified protein expression vector inducible by arabinose	13
pBAD-ldcA	6His-tagged LdcA protein expression vector	This study
pQF50	*bla lacZ* transcriptional fusion vector	4
pQF52	*bla lacZ* translational fusion vector derived from pQF50	23
pST500	*aotJ*::*lacZ* translational fusion of pQF52	22
pZY5	*PA4980*::*lacZ* translational fusion of pQF52	36
pHT1818	*ldcA*::*lacZ* transcriptional fusion of pQF50	This study

[a] Km^r, kanamycin resistance; Sm^r, streptomycin resistance; Sm^s, streptomycin sensitive; Tc^r, tetracyclin resistance; Gm^r, gentamicin resistance; Amp^r, ampicillin resistance.

TABLE 2. Promoter activities of *aotJ* and *ldcA* in response to arginine and lysine

Strain	Description or genotype	Nutrients[a]	Sp act (mmole/mg/min) for:[b]	
			$P_{ldcA::lacZ}$	$P_{aotJ::lacZ}$
PAO1	Wild type	G	0.8 (1)	14 (1)
		A+G	9.5 (12)	61 (4)
		L+G	1.2 (1.5)	18 (1.2)
PAO5715	Δ*aruCFGDBE*::Gm^r	G	4.1 (1)	58 (1)
		A+G	16.0 (4)	105 (2)
		L+G	4.0 (1)	26 (0.5)

[a] Cells were grown in minimal medium P supplemented with 20 mM lysine (L), arginine (A), or glutamate (G).
[b] Specific activities represent the averages from three measurements with standard errors below 5%. Fold changes are in parentheses.

the mid-log phase were harvested by centrifugation and then passed through a French press cell at 8,500 lb/in^2. The cell debris were removed by centrifugation at 20,000 × *g* for 15 min, at 4°C, and protein concentrations in the crude extracts were determined by the Bradford method (1). The levels of β-galactosidase activity were measured using Miller's method (18).

Gel retardation assay. A DNA fragment covering 635 bp upstream of the ATG start codon and the first 24-bp coding sequence of *ldcA* was PCR amplified from *P. aeruginosa* PAO1 chromosome using the designed primers. The DNA probe was labeled with [γ-^{32}P]dATP by polynucleotide kinase. The radioactively labeled DNA probe (0.1 nM) was allowed to interact with different concentrations of the purified ArgR in a 20-μl reaction mixture containing 20 mM Tris-HCl (pH 7.6), 50 mM KCl, 1 mM EDTA, 5% (vol/vol) glycerol, and 50 μg/ml bovine serum albumin. The reaction mixtures were incubated for 15 min at room temperature before applying them to a 5% polyacrylamide gel in Tris-acetate-EDTA running buffer. After being dried, the gel was autoradiographed by exposure to a phosphorimager plate (Fuji).

Overexpression and purification of LdcA. Full-length *ldcA* was amplified from the genomic DNA of *P. aeruginosa* using the following designed primers: 1818F, 5′-TATAAAGACCTCAAATTTCCCGTC-3′; and 1818R, 5′-TCATTCCTTTA TGCATTCAACGGT-3′. The PCR product was subcloned into pBAD-HisD, a modified pBAD expression vector (13, 35). Recombinant 6×His-LdcA was expressed in *Escherichia coli* TOP10 (Invitrogen Life Technologies) by arabinose induction. Cell extract was obtained by passage through a French pressure cell at 8,500 lb/in^2 followed by centrifugation at 25,000 × *g* for 30 min at 4°C. Soluble His-LdcA protein was purified from a HisTrap HP column (GE Healthcare) at the concentration of 100 mM imidazole. Eluted fractions detected by UV were analyzed by SDS-PAGE, pooled together, and concentrated by an Amicon Ultra-15 centrifugal filter unit (Millipore). Active fractions were determined by a lysine decarboxylase assay as described below. Protein concentration was determined by the method of Bradford (1).

Measurements of lysine decarboxylase activity. Purified His-LdcA was used to test L-lysine decarboxylation *in vitro*. Enzyme-catalyzed decarboxylation was assayed by measuring the liberated $^{14}CO_2$ at 37°C as previously described, with slight modification (34). The assay was carried out in a standard scintillation vial, and the liberated $^{14}CO_2$ was trapped by a filter paper preimpregnated with 0.1 ml barium hydroxide in the cap. One milliliter of standard reaction mixture contains 125 μM pyridoxal 5′-phosphate, 100 μg/ml acetylated bovine serum albumin, 0.2 μCi [^{14}C]-L-lysine, and 3 mM cold L-lysine in 100 mM Tris buffer at pH 8.5. The mixture was incubated at 37°C in a water bath, and the reaction was started by adding 1.5 μg of purified LdcA. Reaction was stopped by adding seven drops of 1N H_2SO_4, and the incubation was continued for 30 min more for complete absorption of CO_2. Labeled CO_2 was measured in a liquid scintillation counter. Apparent kinetics parameters were determined using nonlinear regression equations of the kinetics module of SigmaPlot 9.0 software.

L-lysine uptake experiments. Radiolabeled L-lysine was used for uptake assays as previously described, with slight modification (15). Cultures were grown in glutamate-MMP in the absence or presence of 20 mM L-arginine or L-lysine. Cells were harvested during logarithmic growth (optical density at 600 nm [OD_{600}] of 0.5 to 0.8), washed twice, and resuspended at a concentration of ca. 10^8 cells/ml (OD_{600} = 0.1) using MMP containing chloramphenicol (250 μg/ml). After incubation of the cell suspension for 5 min in a 37°C water bath, ^{14}C-labeled L-lysine was added to a final concentration of 20 μM (10 mCi/mmole), and samples (0.5 ml) were withdrawn at various time intervals. Cells were collected on a cellulose membrane filter (0.22-μm-pore size, type GS; Millipore) and washed with 10 ml of MMP. Membranes were air dried in clean scintillation vials overnight. Incorporated radioactivity was measured using adequate scintillation liquid and a spectrometer (Beckman).

RESULTS

ArgR-dependent induction of the *ldcA* promoter by arginine but not by lysine. The putative *ldcAB* operon was initially identified as a member of the ArgR regulon from transcriptome analysis (16). ArgR is autoinducible from the *aot-argR* operon and is initially identified as the major regulator of aerobic arginine catabolism and biosynthesis in response to arginine (23). The *ldcA* promoter in response to exogenous L-arginine and L-lysine was tested by measurements of β-galactosidase activities in PAO1 harboring pHT1818, a $P_{ldcA::lacZ}$ fusion. As shown in Table 2, exogenous arginine but not lysine exerts a strong induction effect (12-fold) on the *ldcA* promoter.

A possible ArgR binding site (from 120 bp to 81 bp upstream of the ATG start codon) was identified previously in the *ldcA* regulatory region (9) based on its sequence similarity to the consensus ArgR binding site (16). Electro-mobility shift assays were conducted to demonstrate the binding of ArgR to the *ldcA* regulatory region. As shown in Fig. 2, ArgR binding to the *ldcAB* regulatory region can be detected using this approach, with an estimated dissociation constant of 1.0 nM.

The *ldcA* gene is essential for lysine utilization. The results of *ldcA* expression in response to arginine from the above-described experiments led us to propose that *ldcA* encodes an arginine-inducible arginine decarboxylase (ADC), the first enzyme of the ADC pathway (21). Arginine succinyltransferase (AST) and arginine transaminase (ATA) pathways were reported to sustain for aerobic growth in *P. aeruginosa* (36). In order to test for the ADC pathway as an alternative route for arginine utilization, we used a double mutant strain, PAO5602, devoid of the AST (by *aruF* deletion) and ATA (by *aruH*::Tc^r) pathways, while the putative ADC pathway remained intact (Table 3). However, we observed that growth on L-arginine as

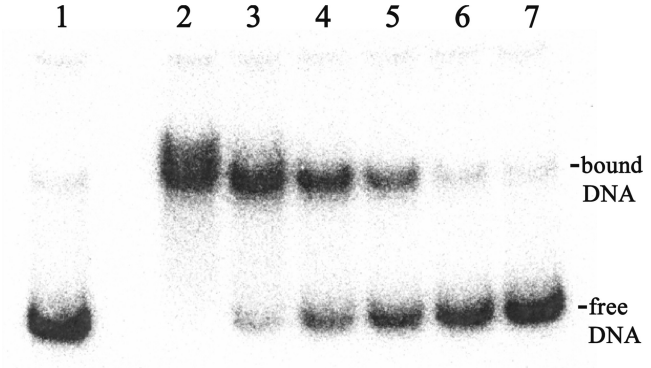

FIG. 2. Gel retardation assay for the binding of ArgR to the *ldcA* promoter. Binding reactions of radioactively labeled regulatory region with different concentrations of purified ArgR were performed *in vitro* and subjected to nondenaturing polyacrylamide gel electrophoresis analysis. DNA probe concentration was 0.1 nM. Protein concentrations from lane 2 to lane 7 were 45 nM, 18 nM, 9 nM, 4.5 nM, 2.2 nM, and 1 nM. Lane 1 served as negative control by removing ArgR from the reaction.

TABLE 3. Lysine decarboxylase pathway is the main route for lysine catabolism

Strain	Description of genotype[b]	Affected pathway	Growth with indicated supplement[a]				
			Lc	Ln	Ac	An	GN
PAO1	WT	None	+	++	+++	+++	+++
PAO5716	*ldcA*::Tc^r	LDC	−	−	+++	+++	+++
PAO5717	*ldcB*::Tc^r	LDC	+/−	++	+++	+++	+++
PAO5720	*aruH*::Tc^r	ATA	+	++	+++	+++	+++
PAO 501	*argR*::Gm^r	AST/LDC	−	+	+	++	+++
PAO5602	*aruH*::Tc^r Δ*aruF*	AST/ATA	++	+++	−	−	+++
PAO5715	Δ*aruCFGDBE*::Gm^r	AST	++	+++	+	++	+++
PAO5718	Δ*aruCFGDBE*::Gm^r *ldcA*::Tc^r	LDC/AST	M	M	+	++	+++

[a] Logarithmically growing cells were plated on MMP solid agar media supplemented with the following: Lc, 20 mM lysine + 5 mM ammonia; Ln, 5 mM lysine + 20 mM glucose; Ac, 20 mM arginine + 5 mM ammonia; An, 5 mM arginine + 20 mM glucose; GN, 20 mM glucose + 5 mM ammonia. Growth at 37°C was recorded daily during 3 days of incubation period as follows: +++, growth in 1 day; ++, growth in 2 days; +, growth in 3 days; +/−, faint growth in 3 days; −, no growth in 3 days; M, growth under stress with high mutation rate.

[b] WT, wild type.

the sole source of carbon and nitrogen was completely abolished in PAO5602 and growth on L-arginine could be restored when the mutant strain was complemented by pHC5307, which expressed *aruH* from the *lac* promoter on the pUCP18 vector (Table 4). Contrarily, PAO5602 harboring pHC5306 (*ldcA* in pUCP18) still showed no growth on L-arginine. From these results we concluded that *ldcA* is not involved in arginine utilization in *P. aeruginosa* PAO1.

Surprisingly, the *ldcA* mutant strain, PAO5716, lost the ability to grow on lysine, while arginine utilization remained unaffected (Table 3). Introducing pHC5306 to PAO5716 complemented the growth defect on lysine. Even growth of the wild-type strain PAO1 on lysine can be enhanced by the presence of pHC5306. These data support that *ldcA* may encode a lysine decarboxylase that is essential for L-lysine catabolism via the decarboxylase pathway (5).

LdcA as lysine-specific PLP-dependent decarboxylase. To further confirm the enzymatic function of LdcA in lysine degradation, we performed an initial characterization of purified His-tagged LdcA with radioactive-labeled L-lysine to determine the reaction rate by measuring the liberated $^{14}CO_2$. As shown in Fig. 3A, LdcA possesses calculated K_m and V_{max} values of 0.73 mM and 2.2μmole/mg/min, respectively. An estimated Hill slope of 2.0 indicated cooperative substrate activation by L-lysine. The decarboxylase activity of LdcA was also checked with ^{14}C-labeled L-arginine as a substrate, and no activity on L-arginine could be detected.

A pyridoxal 5′-phosphate (PLP) binding site of LdcA was predicted for A_{387}THSTHKMLAAF$_{398}$, which shows 90% and 91% similarities to the PLP binding sites of *E. coli* lysine decarboxylase (CadA; ETESTHKLLAAF) and arginine decarboxylase (AdiA; ATHSTHKLLNAF), respectively (20). Indeed, when PLP is removed from the reaction mixture, enzyme activity is abolished for LdcA (data not shown).

The optimal pH and temperature for LdcA were also determined. As shown in Fig. 3C and D, LdcA exhibits a maximum activity around 37°C, pH 8.5, with L-lysine as a substrate. At pH 6, almost no activity was detectable for LdcA in this acidic environment (data not shown). These results strongly suggest that LdcA may not be involved in acid stress as *E. coli* CadA and AdiA are (2, 17, 33) but rather is suitable for lysine catabolism under alkaline conditions.

The potential effects of L-arginine and compounds derived from L-arginine/ornithine/lysine decarboxylation (agmatine, putrescine, and cadaverine) on LdcA were tested. An inhibition effect was detected by the last three compounds but not by L-arginine (Fig. 3B). These results imply that arginine intervention in lysine utilization is exerted only at the transcriptional level and that lysine decarboxylation is subjected to product inhibition by cadaverine and other polyamines.

TABLE 4. Effects of constitutive expression of *ldcA* or *aruH*

Strain	Affected pathway	Plasmid	Description or genotype	Growth with indicated supplement[a]			
				Lc	Ln	Arg	Agm
PAO1	None	pUCP18	WT, pUCP18	+	++	+++	+++
		pHC5307	WT, pUCP18 *aruH*	+	+++	+++	+++
		pHC5306	WT, pUCP18 *ldcA*	+++	+++	+++	+++
PAO5716	LDC	pUCP18	*ldcA*::Tc^r, pUCP18	−	−	+++	+++
		pHC5307	*ldcA*::Tc^r, pUCP18 *aruH*	−	+++	+++	+++
		pHC5306	*ldcA*::Tc^r, pUCP18 *ldcA*	+++	+++	+++	+++
PAO5602	ATA/AST	pUCP18	*aruH*::Tc^r Δ*aruF*, pUCP18	+++	+++	−	+++
		pHC5307	*aruH*::Tc^r Δ*aruF*, pUCP18 *aruH*	+++	+++	+++	+++
		pHC5306	*aruH*::Tc^r Δ*aruF*, pUCP18 *ldcA*	+++	+++	−	+++

[a] Logarithmically growing cells were subjected to growth phenotype test in MMP media supplemented with the following: Lc, 20 mM lysine + 5 mM ammonia; Ln, 5 mM lysine + 20 mM glucose; Arg, 20 mM arginine; Agm, 20 mM agmatine; and GN, 20 mM glucose + 5 mM ammonia. Aerobic growth at 37°C was monitored during 3 days as follows: +++, prominent growth in 1 day; ++, growth in 2 days; +, growth in 3 days; −, no growth in 3 days.

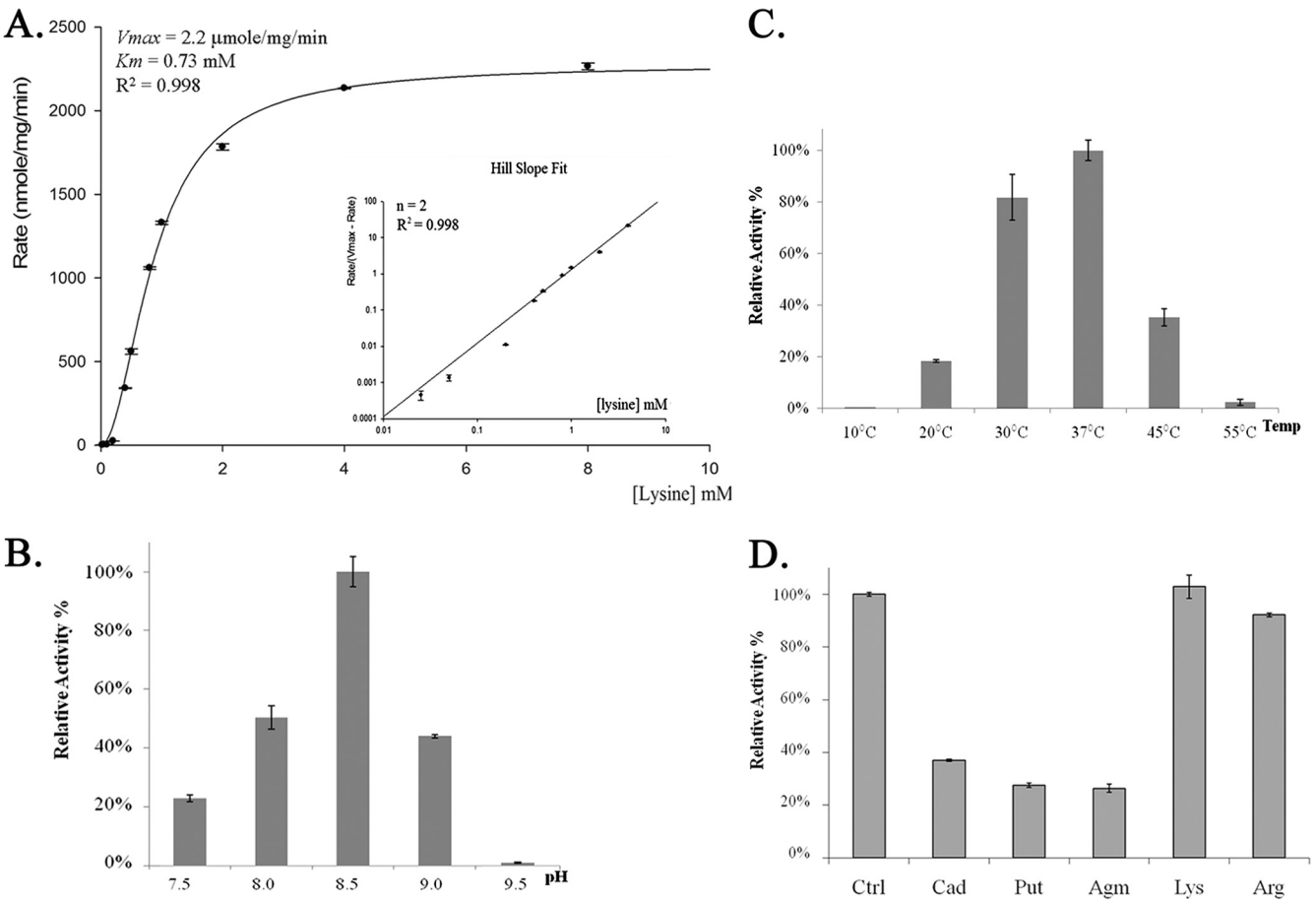

FIG. 3. Lysine decarboxylase enzyme characterization. His-tagged LdcA protein was purified from *E. coli*. Enzyme-catalyzed lysine decarboxylation was assayed by measuring the liberated $^{14}CO_2$ as previously described in Materials and Methods. (A) Kinetics studies exhibiting cooperative substrate activation pattern with the corresponding Hill slope plot. Assays with increasing lysine concentrations were performed at pH 8.5, 37°C. (B and C) Optimal conditions for lysine decarboxylation were tested as described in Materials and Methods. (D) Cold polyamines or amino acids (5 mM) were added into the standard reaction mixture to test for allosteric effects.

Lysine uptake is enhanced by exogenous arginine but not lysine. The arginine effect on lysine utilization may range from uptake to degradation. In order to investigate this hypothesis, lysine uptake was examined in a pair of isogenic strains of *P. aeruginosa*, PAO1 (wild type) and PAO501 (the *argR*::Gm^r mutant). Cells were grown in minimal medium P with L-glutamate as the background nutrient with or without addition of exogenous L-arginine or L-lysine. The uptake of lysine in cell suspensions was examined. As shown in Fig. 4, uptake of lysine is significantly induced by the presence of exogenous arginine but not by lysine. Interestingly, this arginine-inducible lysine uptake is also ArgR dependent (Fig. 4B).

Exogenous arginine improves the growth on lysine in PAO1. All our data indicate that expression of *ldcA*, the essential gene for lysine catabolism, is controlled by ArgR in response to L-arginine but not to L-lysine. In the wild-type strain PAO1, the estimated generation time was about 122 min when grown on L-lysine; however, it experienced a long lag phase of 44 ± 2 h. We reasoned that this growth curve was due to a lack of *ldcA* induction by exogenous lysine and hypothesized that growth of PAO1 on lysine can be improved by a trace amount of L-arginine to kick off *ldcA* expression. Indeed, addition of 0.15 mM L-arginine removed completely the otherwise long lag phase, while the generation time remained unchanged (data not shown).

The basal level of *ldcAB* expression is elevated in the mutant devoid of the major arginine catabolic pathway. In analysis of the growth phenotype of PAO5602 (an arginine nongrower), we observed significant improvement of this strain on lysine in comparison to PAO1 (Table 3). A similar growth behavior on lysine was also detected in PAO5715, an *aruCFGDBE* deletion mutant devoid of the major pathway (AST) for arginine catabolism (11). One hypothesis was that the intracellular level of arginine may be elevated when the major catabolic pathway is blocked, which in turn activates ArgR to induce the ArgR regulon (16), including the *aotJQMOP-argR* operon for uptake and regulation, *ldcAB*, and many others. To test this hypothesis, we compared the activities of the *aotJ* and *ldcA* promoters from *lacZ* fusions in PAO1 and PAO5715. As shown in Table 2, the basal level of these two promoters was increased 4- or 5-fold in PAO5715, and they were still responsive to the presence of arginine but not lysine in both strains. These results support the hypothesis of an activated ArgR regulon and hence high lysine uptake and catabolism in AST mutant strains.

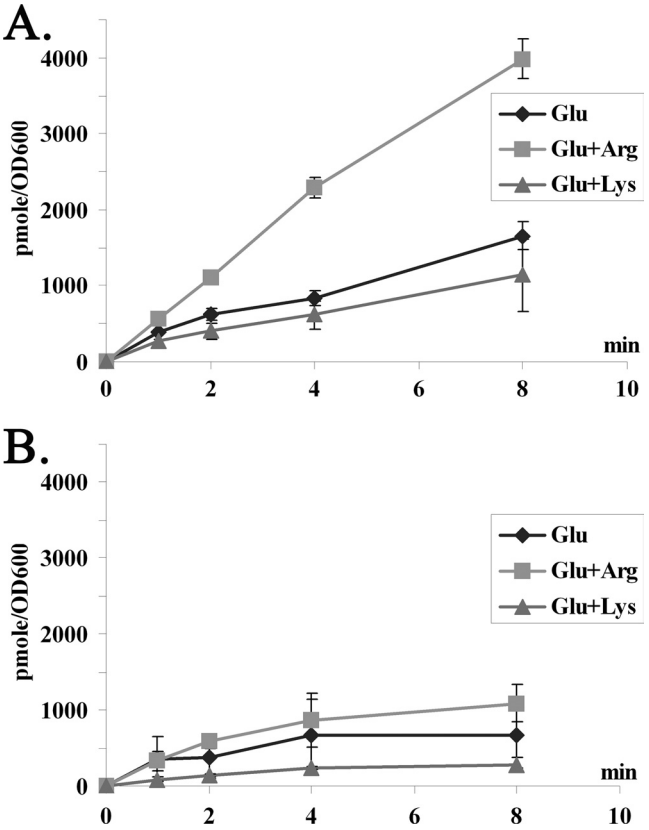

FIG. 4. Enhanced lysine uptake by arginine. Induction of L-lysine uptake by exogenous arginine in *P. aeruginosa* PAO1 (A) and its *argR* mutant (B). Cultures grown in glutamate-MMP in the absence (diamond) or in the presence of L-arginine (square) or L-lysine (triangle) were harvested during exponential growth and used for L-lysine transport assays as described in Materials and Methods.

Potential lysine catabolism by transamination. While the LdcA-catalyzed decarboxylation appeared to be the major route for lysine catabolism, L-lysine can potentially be degraded by other approaches, e.g., transamination. One possible candidate was AruH, which has been studied and characterized *in vitro* as a transaminase able to remove the α-amino group from arginine and lysine (35). Although the *ldcA* mutant lost completely the capability to grow on L-lysine as a sole source of carbon and/or nitrogen (Table 3), introducing *aruH* carried on pHC5307 to this mutant indeed restored growth on lysine as a sole source of nitrogen but not carbon (Table 4).

DISCUSSION

The bottleneck of lysine catabolism. The most intriguing finding in this study was that both lysine uptake and degradation are inducible by L-arginine and show no response to L-lysine. Arginine-dependent *ldcA* expression was revealed from its promoter activities, and electro-mobility shift assays also revealed interactions of the arginine-responsive regulator ArgR to its putative binding site in the promoter region. The lack of lysine-responsive induction on *ldcA* is likely the major limiting factor of growth on L-lysine. Growth of *P. aeruginosa* on L-lysine exhibited a long lag phase of 44 ± 2 h. In comparison, growth on cadaverine, the decarboxylation product of L-lysine by LdcA, had a normal lag phase. Indeed, putting *ldcA* under the control of the constitutive *lac* promoter in a plasmid improved growth significantly.

Conditions that increase the level of intracellular arginine and ArgR could also induce *ldcA* expression and hence better growth on L-lysine. While *ldcA* was induced under these conditions, at least two ABC transporter systems for L-arginine were also increased (*aotJQMP* and *PA5152* to *PA5155*). Interestingly, counterparts of these two systems in *P. putida* KT2440 have been reported as candidate L-lysine transport systems in recent studies (26). *PP0283* to *PP0280* and *PP4486* to *PP4482* show high similarities in gene organization as well as in amino acid sequences (82% and 76%, respectively) with *P. aeruginosa PA5152 to PA5155* and *aotJQMP-argR* loci. Together with LdcB, a candidate lysine/cadaverine antiporter, all three candidate transport systems in *P. aeruginosa* are regulated by ArgR in response to L-arginine but not to L-lysine. This may explain the lack of lysine-inducible uptake as another limiting factor for growth on L-lysine in *P. aeruginosa* PAO1.

The lysine decarboxylase LdcA is conserved among pseudomonads. In the *Pseudomonas* Genome Database (www.pseudomonas.com), orthologs of LdcA can be found in strains of *P. entomophila*, *P. fluorescens*, *P. mendocina*, *P. putida*, and *P. stutzeri*, but not in *P. syringae*. For *P. putida* KT2440, the corresponding gene (*PP4140*) contains an authentic mutation that causes a frameshift of the translated product. While gene organization of *ldcA* and upstream *dnaQ* encoding the epsilon subunit of DNA polymerase III is conserved among these strains, downstream *ldcB* can be found only in strains of *P. aeruginosa*. In conclusion, it is reasonable to predict that most species of pseudomonads possess the L-lysine decarboxylase.

Other pathways of lysine catabolism. The *aruH* gene encodes for an L-arginine and L-lysine:pyruvate transaminase (36). Contribution of this enzyme in lysine utilization as the sole nitrogen source can be detected only when constitutively expressed from a plasmid. However, AruH may not have any physiological implication on lysine catabolism in PAO1, as the *ldcA* mutant showed no growth on L-lysine as the sole source of carbon or nitrogen. Perhaps it was because of low L-lysine uptake and the low affinity of AruH on L-lysine.

The *aruH* and *aruI* genes encode the first two enzymes of the arginine transaminase pathway in *P. aeruginosa* (36). While the *aruHI* genes are conserved in *P. putida*, an extra gene encoding an amino acid racemase is located between *aruH* and *aruI* of this organism. The amino acid sequence of this racemase contains a predicted signal peptide at the N terminus, suggesting its secretion to the periplasm. This implies the role of this additional gene as the racemase to convert L-lysine into D-lysine before being channeled into the D-lysine catabolic pathway in *P. putida* (3, 10, 25).

P. aeruginosa can utilize D-lysine only as a nitrogen source and not as a carbon source by the flavin adenine dinucleotide (FAD)-dependent dehydrogenase DauA (13). Transamination of L-lysine by AruH or D-lysine by DauA leads to α-keto-ε-aminohexanoate, which is spontaneously converted into its cyclic form Δ1-piperideine-2-carboxylate at a physiological pH (12). Recent studies of *P. putida* suggest further degradation of this molecule into L-pipecolate by the reductase DpkA (PP3591) (27). However, viability of this pathway is still unde-

fined in *P. aeruginosa* since the *dpkA* orthologue is not present on the chromosome, the corresponding biochemical reaction cannot be detected, and no growth is detectable using D-lysine as a carbon source (5).

Lysine decarboxylation is the main route of L-lysine catabolism in *P. aeruginosa*. With the purified enzyme, we demonstrated that LdcA is an L-lysine decarboxylase with no activity toward L-arginine. Genetic studies also indicated that *ldcA* is essential for L-lysine utilization in *P. aeruginosa*. Therefore, we concluded that the lysine decarboxylase LdcA catalyzes the pivotal step of L-lysine catabolism in this organism.

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