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Construction of an *Escherichia coli* Strain Unable to Synthesize Putrescine, Spermidine, or Cadaverine: Characterization of Two Genes Controlling Lysine Decarboxylase

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We have previously described a polyamine-deficient strain of *Escherichia coli* that contained deletions in *speA* (arginine decarboxylase), *speB* (agmatine ureohydrolase), *speC* (ornithine decarboxylase), and *speD* (adenosylmethionine decarboxylase). Although this strain completely lacked putrescine and spermidine, it was still able to grow at a slow rate indefinitely on amine-deficient media. However, these cells contained some cadaverine (1,5-diaminopentane). To rule out the possibility that the presence of cadaverine permitted the growth of this strain, we isolated a mutant (*cadA*) that is deficient in cadaverine biosynthesis, namely, a mutant lacking lysine decarboxylase, and transduced this *cadA* gene into the Δ(*speA-speB*) Δ*speC* Δ*speD* strain. The resultant strain had essentially no cadaverine but showed the same phenotypic characteristics as the parent. Thus, these results confirm our previous findings that the polyamines are not essential for the growth of *E. coli* or for the replication of bacteriophages T4 and T7. We have mapped the *cadA* gene at 92 min; the gene order is *mel cadA groE ampA purA*. A regulatory gene for lysine decarboxylase (*cadR*) was also obtained and mapped at 46 min; the gene order is *his cdd cadR fpk gyrA*.

We have previously isolated and described mutants of *Escherichia coli* which have no putrescine (1,4-diaminobutane) or spermidine as a result of deletions in *speA* (arginine decarboxylase), *speB* (agmatine ureohydrolase), *speC* (ornithine decarboxylase), and *speD* (adenosylmethionine decarboxylase) (7). Despite this amine deficiency, these strains grew indefinitely at a growth rate one-third of that found in the presence of polyamines. Even though bacteriophage λ production was markedly inhibited, the amine-deficient strains could act as hosts for bacteriophages T4, T7, and f2.

Since these strains contained (7) a small amount of cadaverine (1,5-diaminopentane), it was possible that this amine permitted the indefinite, albeit slow, growth of the amine-deficient strain and the production of bacteriophages T4, T7, and f2. Consequently, we searched for a mutant that was defective in the biosynthesis of this amine, i.e., lacking lysine decarboxylase activity. Such a mutation was obtained and, after mapping, was introduced into the strain already amine deficient because of deletions in *speA, speB, speC,* and *speD*. The resultant strain had only traces of cadaverine when grown on an amine-deficient medium. This strain did not show any phenotypic differences from the parent Δ(*speA-speB*) Δ*speC* Δ*speD* strain, i.e., from the strain only lacking putrescine and spermidine.

During this work we also isolated and mapped a regulatory gene for lysine decarboxylase (*cadR*).

(A preliminary report on the isolation of mutants that overproduce lysine decarboxylase and that are defective in the production of this enzyme was presented at the 11th International Congress of Biochemistry in Toronto, 13 July 1979 [C. W. Tabor, E. W. Hafner, and H. Tabor, Abstr. no. 07-8H82].)

MATERIALS AND METHODS

Methods for mutagenesis (with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine), mapping (P1 transduction and Hfr crosses), bacterial growth, and phage replication were as described previously (7, 14) or as described by Miller (10). The strains used are listed in Table 1. Minimal medium was that described by Vogel and Bonner (17); inducing medium was essentially that of Falkow as described by Skerman (18) and contained (per liter) 5 g of peptone (Difco Laboratories), 3 g of yeast extract, 1 g of glucose, and 10 g of DL-lysine.

Assays in the mapping experiments were based on the inability of *groE* strains to grow at 44°C (5, 6), the resistance of *ampA* strains (6) to 10 μg of ampicillin per ml on LB plates, the ability of *fpk*$^+$ (9) and *cdd*$^+$

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TABLE 1. *Strains used*

Strain	Genotypic characteristics
HT297[a]	*ara leu lacY purE gal trp his argG malA rpsL xyl mtl ilv thi gyrA fpk ampA cadA cadR*
HT302	*ara leu lacY purE gal trp his argG malA rpsL xyl mtl ilv thi gyrA cadA cadR*
HT306	*thr-1 proA2 thi-1 lacY1 galK2 mtl-1 xyl-5 ara-14 rpsL-25 supE44 cadA ampA Δ(speA-speB) Δ(speC-glc) ΔspeD*
HT316	*ara leu lacY purE gal trp his argG malA rpsL xyl mtl ilv thi gyrA fpk-1 cadR*
HT337	*thr-1 leu-6 thi-1 his-1 purA45 pro27 xyl-7 malA1 ara-13 gal-6 lacY1 rpsL9 tonA2 gyrA cadR*
HT348	*ara leu lacY purE gal trp his argG malA rpsL xyl mtl ilv thi gyrA fpk-1 cadR*
HT360[b]	*thr-1 leu-6 thi-1 his-1 pro27 xyl-7 malA1 ara-13 gal-6 lacY1 rpsL9 tonA2 gyrA cadR groE*
Sφ423[c]	*his-205 udk-2 cdd-5 ufp-11 relA1 metB1 rpsL254*
EWH319	*thr-1 proA2 thi-1 lacY1 galK2 mtl-1 xyl-5 ara-14 rpsL-25 supE44 Δ(speA-speB) Δ(speC-glc) ΔspeD*
CSH57[a]	*ara leu lacY purE gal trp his argG malA rpsL xyl mtl ilv thi*

[a] *cadR* was originally obtained by mutagenesis (see text) of CSH57 (from the Cold Spring Harbor Collection [10]). *fpk* (originally obtained [9] as AT 2243.11[c].25) was transferred to this *cadR* strain by cotransduction with *gyrA* (cotransduction frequency, 0.5 to 1%). *cadA* was introduced by cotransduction with *ampA*.

[b] A *groE* strain was originally obtained from C. P. Georgopoulos (5).

[c] Reference 8.

(2) strains to grow on 0.04% fructose (2.2 mM) or 0.44% cytidine (18 mM) as the sole source of carbon on minimal agar (17) plates (containing, in addition, the minimum auxotrophic requirements for the strains being tested), and the auxotrophic requirement of *purA* strains for adenine.

Amines were assayed by the automated column method described previously (14).

Qualitative assays (in vivo) for lysine decarboxylase were carried out in Falcon plates (Falcon Plastics) by a modification of the method that we previously developed for other decarboxylase reactions (16). Single clones were grown overnight in Vogel-Bonner minimal medium (17) containing the auxotrophic requirements for the strain and 0.05% glucose at 37°C (volume of fluid in each well, 100 μl). Additional glucose in Vogel-Bonner minimal medium was then added (final concentration, 0.4%), and incubation at 37°C was continued for about 4 h. DL-[1-^{14}C]lysine (10,000 cpm, 1.4 nmol) was then added to each well in a volume of 25 μl. A $Ba(OH)_2$-impregnated filter paper was placed over each plate as described previously, and incubation was continued for 4 to 12 h. The paper was then removed, dried, and subjected to radioautography to score for the production of $^{14}CO_2$ by the culture contained in each well.

Lysine decarboxylase was assayed in vitro by a modification of the method previously described for ornithine decarboxylase (15). In the experiment with noninducing medium (Table 2), cells were grown with aeration in 50 ml of Vogel-Bonner medium plus 0.4% glucose, the required amino acids, and L-lysine (7×10^{-4} M) at 37°C to a cell density of 3×10^9 cells per ml. In the experiment with inducing medium (Table 2), the cells were first grown to stationary phase in Luria broth medium at 37°C. The cultures were then diluted threefold into Falkow inducing medium (18) and incubated at 37°C with shaking for 1.5 h and then without shaking for 4 h. In both cases, the cells were harvested by centrifugation and suspended in 2.5 to 5 ml of 10^{-3} M dithiothreitol–10^{-3} M EDTA (pH 7.2)–10^{-4} M pyridoxal phosphate; the suspension was treated with toluene (11) for 30 min at 37°C. Lysine decarboxylase activity was assayed in vials (7, 15) by incubating 0.25-ml aliquots of this suspension with 0.1 ml of a mix containing 0.12 M sodium acetate (pH 5.2), 1.8×10^{-3} M L-lysine, 2.0×10^5 cpm of DL-[1-^{14}C]-lysine, 2×10^{-4} M pyridoxal phosphate, and 0.2 mg of bovine serum albumin. The $^{14}CO_2$ released was trapped on filter paper (containing Hyamine hydroxide) in the lids and counted in a scintillation counter.

RESULTS AND DISCUSSION

To obtain a strain that did not have any cadaverine, we searched for a mutant lacking lysine decarboxylase. Since we assumed that no auxotrophic requirement for cadaverine would result from defects in lysine decarboxylase, we decided to assay single-colony isolates from a mutagenized culture for such defects by the in vivo formation of $^{14}CO_2$ from [^{14}COOH]lysine. However, this posed a problem as *E. coli* normally has very little lysine decarboxylase activity when grown on a minimal medium. Since it is well known that lysine decarboxylase is an inducible enzyme (13), we decided to first select a mutant that overproduced lysine decarboxylase, i.e., a mutant presumably defective in a

TABLE 2. *Effect of cadA and cadR mutations on lysine decarboxylase activity in toluene-treated cells*

Medium	Strain	Relevant genotypes	Lysine decarboxylase activity[a]
Noninducing	CSH57	*cadA*$^+$ *cadR*$^+$	60
	HT316[b]	*cadA*$^+$ *cadR*	35,000
	HT302[b]	*cadA cadR*	60
Inducing	CSH57	*cadA*$^+$ *cadR*$^+$	175,000
	HT316	*cadA*$^+$ *cadR*	148,000
	HT302	*cadA cadR*	610

[a] Counts per minute of $^{14}CO_2$ released per 30 min per 10^{10} cells.

[b] HT302 and HT316 are derivatives of CSH57.

regulatory gene. We found such a mutation after mutagenesis of strain CSH57 with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and called it *cadR*.

This *cadR* strain was then mutagenized again, and a mutant was obtained that was deficient in lysine decarboxylase due to a mutation distant from *cadR* (see below). We have designated this locus *cadA*.

The lysine decarboxylase activity of these mutants and of the parent strain are presented in Table 2.

Mapping of *cadA* and *cadR*. *cadA* was first located in the *ampA* region by Hfr crosses. Three-point crosses showed the order to be *cadA groE ampA purA* (Table 3, Fig. 1).

cadR was first located between *gyrA* (*nalA*) and *his* by Hfr crosses. Subsequent P1 transduction of *cadR* showed a 27% cotransduction with *fpk*. No cotransduction was found with *gyrA*. A three-point cross showed the order to be *cdd cadR fpk* (Table 4, Fig. 2). The *cadR* gene reported in this paper may be identical to the *lysP* gene recently reported by Popkin and Maas (12). The *lysP* mutants were selected as spontaneous *S*-aminoethylcysteine (thiosine)-resistant organisms; these mutants had a decreased

TABLE 3. *Mapping of cadA by P1 transductions (order: cadA groE ampA purA)*

Cross	Selected marker	No.	Recombinants	No.
1. HT297 *cadA ampA* + × HT337[a] + + *purA*	*purA*$^+$	140	*purA*$^+$ *cadA ampA*	9
			purA$^+$ *cadA*$^+$ *ampA*	19
			purA$^+$ *cadA*$^+$ *ampA*$^+$	112
			purA$^+$ *cadA ampA*$^+$	0[b]
2. HT297 *cadA* + *ampA* × HT360[a] + *groE* +	*ampA*[c]	236	*ampA cadA groE*$^+$	123
			ampA cadA groE	0[d]
			ampA cadA$^+$ *groE*	26
			ampA cadA$^+$ *groE*$^+$	87
	groE$^+$[c]	95	*groE*$^+$ *cadA ampA*	23
			groE$^+$ *cadA ampA*$^+$	6
			groE$^+$ *cadA*$^+$ *ampA*$^+$	24
			groE$^+$ *cadA*$^+$ *ampA*	42

[a] Both HT337 and HT360 contained *cadR* (i.e., both strains were derepressed for lysine decarboxylase).
[b] A double crossover would be required to generate this recombinant.
[c] All incubations were at 30°C, since *groE* strains are temperature sensitive for growth (5).
[d] A double crossover would be required to generate this recombinant. The designation of *ampA*, rather than *groE*, as the outside marker is based on the data of Guest and Nice (6).

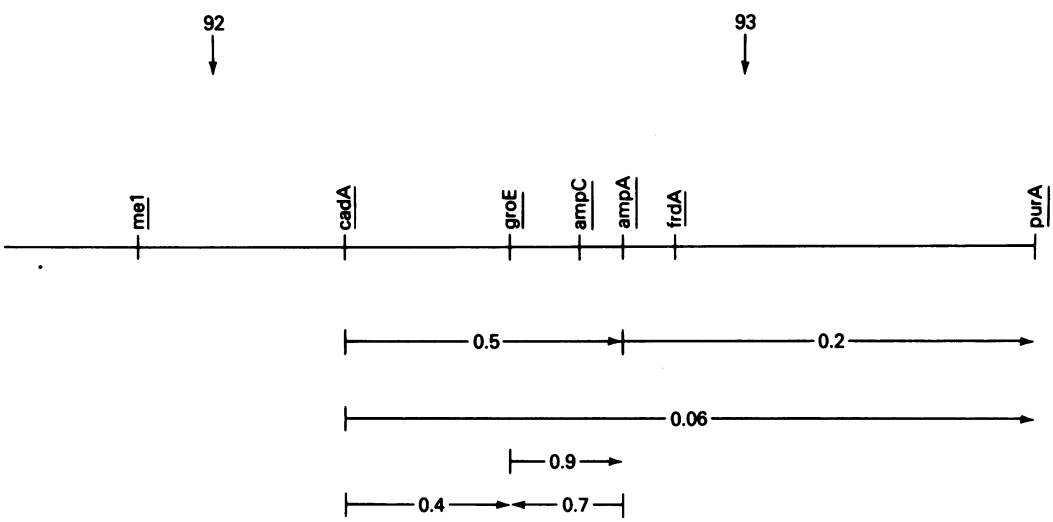

FIG. 1. *Cotransductional frequencies of cadA, groE, ampA, and purA. The position of the other markers is taken from Guest and Nice (6) and from Bachmann and Low (1). The numbers above the map indicate minutes on the E. coli map. The numbers below the map indicate cotransductional frequencies, with the head of the arrow representing the selected marker.*

TABLE 4. *Mapping of cadR by P1 transductions (order: cdd cadR fpk)*

Cross	Selected marker	No.	Recombinants	No.
1. Sφ423 *cdd* + + × HT348 + *cadR fpk*	*fpk*$^+$	138	*fpk*$^+$ *cadR*$^+$ *cdd*	21
			fpk$^+$ *cadR*$^+$ *cdd*$^+$	16
			fpk$^+$ *cadR cdd*$^+$	92
			fpk$^+$ *cadR cdd*	9[a]
2. HT297 + *cadR fpk* × Sφ423 *cdd* + +	*cdd*$^+$	92	*cdd*$^+$ *cadR fpk*	23
			cdd$^+$ *cadR fpk*$^+$	25
			cdd$^+$ *cadR*$^+$ *fpk*$^+$	44
			cdd$^+$ *cadR*$^+$ *fpk*	0[a]

[a] A double crossover would be required to generate this recombinant.

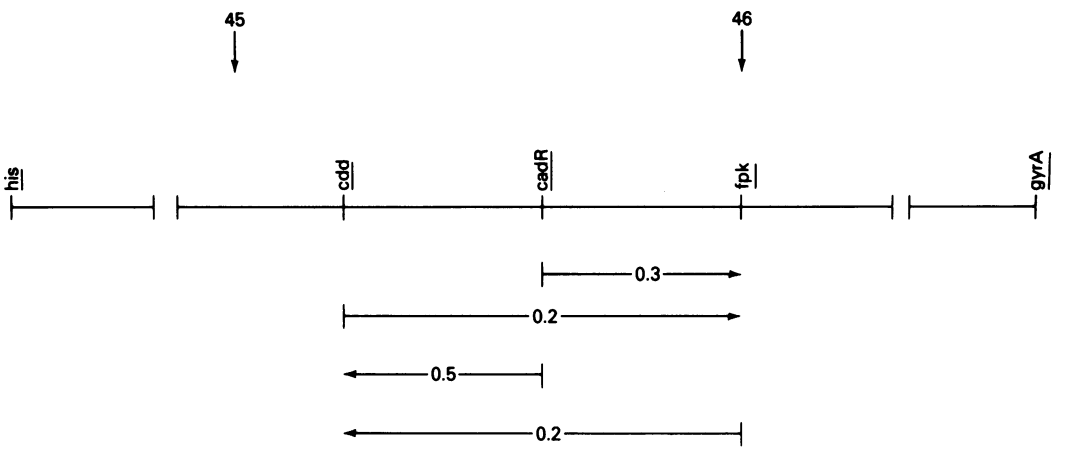

FIG. 2. *Cotransductional frequencies of cdd cadR fpk.*

transport of lysine and derepressed levels of lysine decarboxylase. Popkin and Maas located *lysP* between *gyrA* and *metG* by conjugative crosses. However, more detailed mapping was not done.

We have found that the *cadR* mutant is also resistant to *S*-aminoethylcysteine (tested at 25 μg/ml). *cadA cadR* strains are still resistant to *S*-aminoethylcysteine, confirming the conclusion of Popkin and Maas (12) that resistance to this analog is not the result of its decarboxylation by the increased lysine decarboxylase.

Construction of Δ(*speA-speB*) Δ*speC* Δ*speD cadA* strain. We constructed a quintuple mutant (strain HT306) deficient in cadaverine, putrescine, and spermidine biosynthesis by a P1 cotransduction of *cadA* with *ampA* into the Δ(*speA-speB*) Δ*speC* Δ*speD* strain (EWH319) that we recently described (7). This strain contained <0.02 μmol of cadaverine per g (wet weight). The parent Δ(*speA-speB*) Δ*speC* Δ*speD cadA*$^+$ strain contained 0.2 μmol of cadaverine per g (wet weight). (Wild-type *E. coli* strains, harvested during logarithmic growth, do not usually contain any cadaverine when grown on purified media. Dion and Cohen [3] first showed that cadaverine levels increase when mutants that are partially deficient in amine biosynthesis are grown in the absence of amines.)

Despite the absence of cadaverine, the new strain (HT306) grew at essentially the same rate as its Δ(*speA-speB*) Δ*speC* Δ*speD* parent (EWH319) in a purified medium in the absence of added amines (Fig. 3). In amine-deficient media strain HT306 was able to serve as a host for bacteriophages T4 and T7, although the phage yield was somewhat lower; these findings are similar to those previously described for the Δ(*speA-speB*) Δ*speC* Δ*speD* strain (7). The bacteriophage T4 and T7 preparations used in these experiments had been prepared by growth in the deficient strain (HT306). All of these experiments and the phage assays were carried out under the conditions described in reference 7.

These findings indicate that the ability of the Δ(*speA-speB*) Δ*speC* Δ*speD* mutant to grow and to maintain phage is not attributable to the cadaverine in these mutants. Furthermore, the absence of cadaverine in the Δ(*speA-speB*) Δ*speC* Δ*speD cadA* mutant is not associated with any obvious phenotype.

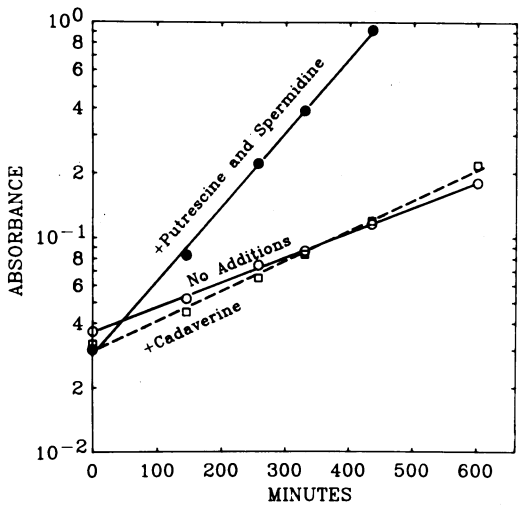

FIG. 3. *Growth of the Δ(speA-speB) ΔspeC ΔspeD cadA strain (HT306) in the presence and absence of amines. Strain HT306 was grown in minimal medium (plus glucose) for at least 13 generations and then divided into three portions: (i) no addition; (ii) 2.5 × 10^{-5} M cadaverine; and (iii) 2.5 × 10^{-5} M putrescine and 2.5 × 10^{-6} M spermidine. After 18 h of additional incubation with shaking at 37°C, each culture was diluted into the same medium to give an absorbance at 540 nm of approximately 0.03, and the incubation was continued.*

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ADDENDUM

Since this manuscript was completed, S. H. Goldemberg (J. Bacteriol. **141**:1428–1431, 1980) reported two mutants with a very low lysine decarboxylase, even under inducing conditions. No mapping studies were carried out.

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