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Escherichia coli Regulatory Mutation Affecting Lysine Transport and Lysine Decarboxylase

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A spontaneous thiosine-resistant mutant of *Escherichia coli* was shown to have the following characteristics: lowered initial rate of lysine uptake and lowered plateau level of accumulation of exogenous lysine by both the lysine-specific and the general basic amino acid transport systems; altered repressibility of these two lysine transport systems; a derepressed level of lysine decarboxylase; normal growth rate; parental levels of lysyl-transfer ribonucleic acid synthetase and the inducible and constitutive arginine and ornithine decarboxylases. Both the mutant (*lysP*) and its parent (*lysP*$^+$) feed a lysine auxotroph when they are plated in proximity on solid medium. However, the feeding response was observable after 1 day less of incubation when the mutant was the feeding strain. Despite the derepressed level of lysine decarboxylase in exponential cultures of the mutant, extracts of these cultures had no detectable cadaverine pool. Conjugation experiments established the following gene order: *gyrA* (formerly *nalA*) *lysP metG his*. All thiosine-resistant recombinants assayed showed reduced lysine transport. In many of these recombinants the derepression of lysine decarboxylase was not expressed.

Thiosine (*S*-aminoethyl cysteine; also known as thialysine) is a lysine analog that inhibits the growth of a variety of microorganisms in the absence of exogenous lysine. Thiosine participates in many cellular reactions. It has been shown to replace lysine in pyrophosphate exchange and in the aminoacylation of tRNA in the presence of *Escherichia coli* lysyl tRNA synthetase (28), to both inhibit and repress the lysine-specific aspartokinase of *Salmonella typhimurium* (6), and to inhibit lysine uptake by *E. coli* without affecting ornithine uptake (24). Thiosine is decarboxylated by purified *E. coli* lysine decarboxylase at 15 to 20% of the rate at which lysine is decarboxylated by this enzyme (25).

Mutants of yeast resistant to thiosine and with altered lysine-specific transport have been described (13). In bacteria thiosine-resistant mutants have been found which exhibit altered growth medium-dependent levels of lysyl tRNA synthetase (17, 18), the absence of the threonine-controlled aspartokinase-homoserine dehydrogenase complex activity in extracts (19), derepression of aspartokinase III (29), feedback densensitization of the lysine biosynthetic pathway at either of the two normally feedback-sensitive enzymes (aspartokinase or dihydrodipicolinic acid synthetase) (7, 15, 26), and decreased lysine transport by both the lysine-specific system and the general basic amino acid transport system for lysine, arginine, and ornithine (LAO system) (14).

We found that thiosine-resistant mutants of *E. coli* K12 strain JC182 occur spontaneously in minimal medium cultures at high frequencies, and all such mutants examined had lowered lysine transport. Several of the mutants are pleiotropic and show, in addition to lowered lysine transport, derepressed levels of lysine decarboxylase. This pleiotropic genetic defect maps about midway between the *his* and *gyrA* (formerly *nalA*) loci on the *E. coli* chromosome. Our results suggest that a single locus, designated *lysP*, is involved, which codes for a regulatory unit controlling both lysine transport and lysine decarboxylase. We present a model of how such a regulatory entity might function, which is consistent with all experimental findings

MATERIALS AND METHODS

Bacterial strains. Table 1 lists the bacterial strains used in this study.

Media and growth of strains. Unless otherwise specified, strains were grown in minimal medium A (9) supplemented with 0.5% glucose and the particular auxotrophic requirements of the strain. Amino acids were added to a concentration of 100 μ/ml, along with thiamine at 1 μ/ml, adenosine at 40 μ/ml, thymine at 50 μ/ml, *p*-hydroxybenzoic acid at 10 μ/ml, and *p*-aminobenzoic acid at 10 μ/ml. The optical densities of cultures were measured at 490 nm with a Lumetron colorimeter. Exponential cultures were harvested at

TABLE 1. *Description of E. coli strains*

Designation	Genetic characteristics	Source, origin, or previous designation
JC182	*thi purF* double male	A. J. Clark
JC182-5	*thi purF argP* double male	
PSP01	*thi purF lysP* double male	
MA140	*thi thr leu argE pro his trp thyA rpsL*	AB2495
220Ag	*thi his try serA argA*	
MA74	*thi arg pro his aroD rpsL*	AB1359
Hfr44	Hfr *ilv argA argS argR*	P. H. Cooper
KL96	Hfr	B. Low
KL16	Hfr *thi*	B. Low
KL98	Hfr	CGSC[a]
KL166	Hfr *thi thyA gyrA*	CGSC
KLF29/1553	F' *his*$^+$/*his argG metB leu recA rpsL*	CGSC
PSP100	*thi arg aroD rpsL lysP*	PSP01 × MA74
PSP121	*thi thr leu arg pro trp his thy rpsL lysP*	PSP01 × MA140
SB1803	*metG thr leu his proA rpsL*	T. Blumenthal
MA31	*thi leu argG his trp met rpsL*	JC1552
MA255	*thi thr leu speA speC rpsL*	S. Cunningham-Rundles

[a] CGS, Coli Genetic Stock Center, Yale University, New Haven, Conn.

an optical density of 0.3 U, which is equivalent to 6×10^8 cells per ml. For selection and scoring of thiosine-resistant mutants, the minimal medium was supplemented with a lysine-free mixture of amino acids. This supplement was modified from that described by Maas (22) by the omission of lysine and the addition of L-serine (50 μ/ml) and L-arginine (100 μ/ml). In this medium 10 μg of thiosine (Cyclo) per ml inhibits the growth of JC182. However, 25 μg of thiosine per ml was used routinely because other *E. coli* strains were tested and found to be less sensitive to thiosine than JC182.

Falkow medium was described by Skerman (27). Agar (Difco) at a concentration of 2% was added to liquid media to prepare solid media.

Bacteriophage P1 were grown and transduction was carried out as described by Glansdorff (12).

Transport assays. Cells were grown, harvested, washed, preincubated, and assayed for ability to take up radioactive amino acids as described by Celis et al. (4) for their initial rate experiments. For measurement of the plateau level of uptake, cells were incubated for 5 min with a radioactive amino acid. For initial rate determinations, the incubation time was 15 s.

Enzyme assays. For decarboxylase assays cells were harvested and extracted as described by Hirschfield et al. (16). The reaction mixtures for the lysine decarboxylase assays contained (in a total volume of 0.5 ml) 0.1 ml of cell extract, 1.2 μmol of $MgSO_4$, 1.6 μg of pyridoxal phosphate, 12 μmol of [^{14}C]lysine (specific activity, 0.05 μCi/μmol), and 80 μmol of acetate buffer, pH 5.6. Reaction mixtures for the inducible arginine decarboxylase, the constitutive arginine decarboxylase, the inducible ornithine decarboxylase, and the constitutive ornithine decarboxylase were as described by Leifer (Z. Leifer, Ph.D. thesis, New York University, New York, N.Y., 1972). Reactions were carried out at 37°C in scintillation vials, into the caps of which were fitted filter paper cones impregnated with 0.1 ml of NCS reagent (Amersham/Searle), a CO_2-trapping compound. At the end of the incubation period, the reaction was stopped with 0.2 ml of 1 N H_2SO_4. Caps were replaced, and the vials were kept at 37°C for an additional 30 min for absorption of residual CO_2. Filters were then placed in Liquifluor scintillation fluid for counting. A background sample in which the resuspending buffer replaced the cell extract was always run, and its value was subtracted. Activity was linear for 60 min. Incubation time was most commonly 30 min. Lysyl tRNA synthetase was assayed as described by Mehler and Stern (23). Protein was determined by the method of Lowry et al. (21).

Polyamine pools. Cultures were extracted and analyzed by the method of Dion and Cohen (10).

RESULTS

Isolation of mutant PSP01. A minimal medium culture of JC182 was streaked onto two lysine-free plates supplemented with thiamine and adenosine with and without 100 μg of thiosine per ml. After overnight incubation at 37°C, there was full growth on plates without thiosine and no growth except for the appearance of discrete thiosine-resistant colonies on the thiosine-containing plates. A total of 15 of these colonies were purified, and all were found to have lowered lysine transport and high levels of lysine decarboxylase. One of these mutants, PSP01, was chosen for further study and is described below. In another experiment 12 thiosine-resistant mutants were isolated on lysine-free medium supplemented with thiamine, adenosine, and 25 μg of thiosine per ml. All 12 of these thiosine-resistant clones showed lowered lysine transport, but only 4 of the 12 showed the very high lysine decarboxylase levels characteristic of the first set of mutants isolated.

Lysine transport in JC182 and thiosine-resistant mutant PSP01. Lysine uptake was measured in exponential cells at both 10^{-5} and 10^{-7} M lysine. Uptake at the former concentration measures primarily transport by the lysine-specific transport system (K_m, 5.0×10^{-6} M), whereas uptake at the latter concentration measures lysine transport by the LAO system (K_m for lysine, 1.0×10^{-7} M). Figure 1 shows the time course of [^{3}H]lysine uptake by JC182 and PSP01. Table 2 shows the initial rates of [^{3}H]lysine and [^{3}H]arginine uptake.

Arginine uptake was measured in both mutant and parent strains at 10^{-6} and 10^{-8} M [^{3}H]arginine. The initial rates and plateau levels of arginine accumulation were found to be unaltered

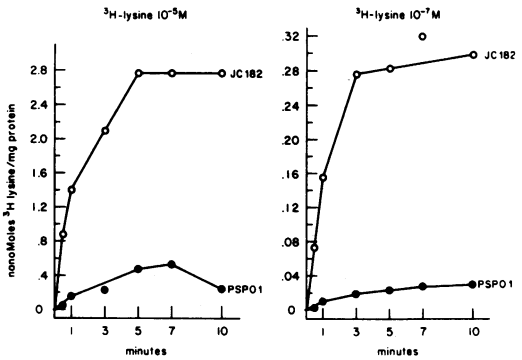

FIG. 1. *Uptake of [³H]lysine by strain JC182 and the thiosine-resistant mutant PSP01. Cells were grown in minimal medium and harvested during the exponential phase of growth.*

TABLE 2. *Initial rates of basic amino acid uptake*

Initial external concn		Rate of uptake (nmol/min per mg of protein)	
Compound	Concn (μM)	JC182	PSP01
[³H]lysine	10	2.09	0.38
[³H]lysine	0.1	0.15	0.03
[³H]arginine	1	0.59	0.69
[³H]arginine	0.01	0.05	0.07

in the mutant at both concentrations. The normal arginine transport at 10^{-6} M [³H]arginine in the mutant indicates that this mutation does not affect the arginine-specific transport system (K_m 1.25×10^{-7} M) and that the lowered lysine transport in the mutant is not due to a general transport defect. The normal arginine transport at 10^{-8} M [³H]arginine supports the conclusion of Rosen (24) that the LAO system (K_m for arginine, 5.0×10^{-9} M), although capable of binding arginine, contributes little to arginine transport. Alternatively, only the lysine transport function of the LAO system might be affected.

All transport assays were performed in the presence of 20 mM glucose, 80 μg of chloramphenicol per ml, and 8 mM aminooxyacetic acid. The chloramphenicol prevented the flow of radioactive amino acids into protein. Aminooxyacetic acid, a pyridoxal phosphate inhibitor (30), was added to prevent the decarboxylation of lysine and arginine to cadaverine and putrescine, respectively, by the pyridoxal phosphate-dependent decarboxylases. In the arginine system this inhibition is clearly necessary if one wishes to measure arginine transport because of the constitutive arginine decarboxylase activity (30). In the measurement of lysine uptake, the necessity for aminooxyacetic acid is less obvious, as observed by Rosen (24) and confirmed by our observation of the absence of a measurable cadaverine pool in exponential cultures of both the mutant and its parent (see below).

Lysine decarboxylase levels. Lysine decarboxylase in *E. coli* is an inducible enzyme (11). Maximal enzyme synthesis is achieved under growth conditions of low pH, low aeration, and high concentrations of lysine and glucose (25). Therefore, lysine decarboxylase activity was measured in both exponential- and stationary-phase cells in both minimal medium and Falkow medium (a nonbuffered inducing medium containing 0.5% L-lysine). Table 3 shows a derepression of lysine decarboxylase of approximately 18-fold in exponential cultures of the mutant growing in minimal medium. If the cells were allowed to grow overnight, there was no change in the lysine decarboxylase level of the wild type, but there was an additional more than 10-fold derepression in the mutant. Both strains grown in the inducing medium showed about the same level of lysine decarboxylase. As previously observed by Sabo et al. (25), the enzyme level in cells grown in inducing medium is lower in stationary cells than in exponential cells.

Independence of the lysine transport and lysine decarboxylase defects. It was possible to establish the physiological independence of the lysine transport and lysine decarboxylase defects in the mutant by measuring lysine uptake in cells grown in Falkow medium, in which both the parent and the mutant strains have approximately the same lysine decarboxylase level (Tables 3 and 4). If the lowered lysine uptake observed in the mutant were a consequence of the increased lysine decarboxylase activity, the difference in uptake between the

TABLE 3. *Lysine decarboxylase levels*

Strain	Growth medium	Enzyme activity[a]	
		Exponential cells	Stationary cells
JC182	Minimal	2.4	2.2
PSP01	Minimal	39.5	482
JC182	Falkow	170	114
PSP01	Falkow	233	87

[a] Enzyme activities are expressed in nanomoles of CO_2 per minute per milligram of protein.

TABLE 4. *Plateau level accumulation of lysine by cells grown in Falkow medium*

Initial external lysine concn (M)	Uptake (nmol/mg of protein)	
	JC182	PSP01
10^{-5}	1.21	0.13
10^{-7}	0.12	0.005

two strains would not have persisted under these growth conditions. Note by comparison with the plateau values of Fig. 1 that the level of lysine accumulation is lower in cells grown in Falkow medium than in cells grown in minimal medium. This is due to the repression of lysine transport in cells grown in the presence of lysine (4). We confirmed the repression of lysine transport by lysine by comparing the uptake of lysine in cells grown in minimal medium with its uptake in cells grown in minimal medium plus 100 μg of L-lysine per ml.

Altered repressibility of lysine transport in PSP01. In minimal medium containing 100 μg of L-lysine per ml there was a greater repression of the lysine transport systems than the repression observed by Celis et al. (4) in cells grown in HALF medium containing 1,000 μg of L-lysine per ml (Fig. 2). The lysine-specific system was repressed 3.3-fold, and the LAO system was repressed 4.0-fold when JC182 was grown in minimal medium containing 100 μg of L-lysine per ml. Growth of the mutant PSP01 in the presence of 100 μg of L-lysine per ml did not significantly repress the already lowered lysine transport activities.

Cadaverine pools. Cadaverine is the product of lysine decarboxylation. Wild-type *E. coli* cells have no measurable cadaverine pool unless they are grown in a medium that induces lysine decarboxylase (10; Leifer, Ph.D thesis). Polyamine auxotrophs blocked in the synthesis of putrescine accumulate cadaverine in the absence of exogenous lysine (10).

It was of interest to examine the cadaverine pool of our mutant to determine whether the cadaverine pool reflects the derepression of lysine decarboxylase. Photographs of thin-layer chromatographic analyses of the polyamine pools are shown in Fig. 3. Exponential cells of both the mutant and its wild-type parent had no detectable cadaverine pool (Fig. 3A). However, when the polyamine pools of stationary cultures were analyzed, we saw a cadaverine pool in the mutant, but not in its wild-type parent (Fig. 3B).

Effect of the *lysP* mutation on the growth rate of a polyamine auxotroph. Mutants blocked in putrescine synthesis are capable of

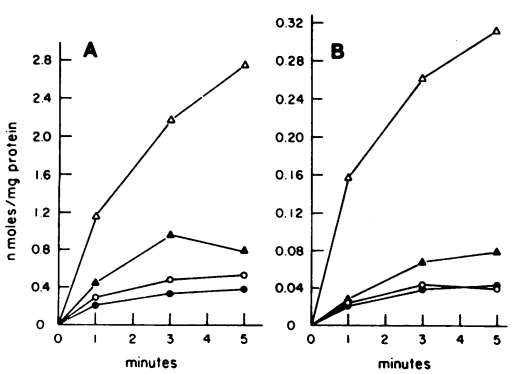

FIG. 2. *Effect of pregrowth in the presence of 100 μg of L-lysine per ml on [^{3}H]lysine uptake in JC182 and PSP01. Initial external lysine concentrations were 10^{-5} M (A) and 10^{-7} M (B). Symbols: △, JC182 grown in minimal medium; ▲, JC182 grown in minimal medium plus 100 μg of L-lysine per ml; ○, PSP01 grown in minimal medium; ●, PSP01 grown in minimal medium plus 100 μg of L-lysine per ml.*

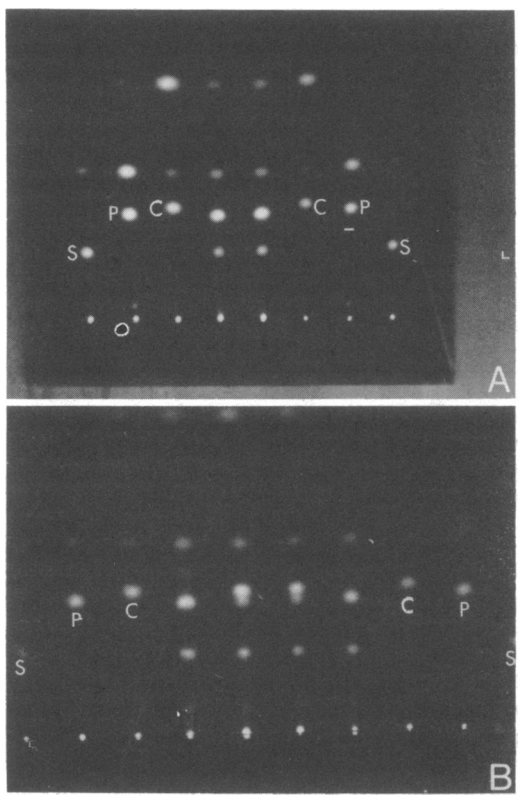

FIG. 3. *Thin-layer chromatograms of dansyl polyamine derivatives of perchloric acid cell extracts of JC182 and PSP01. The polyamine pools of exponential minimal medium cultures are shown in (A), and those of stationary minimal cultures are shown in (B). Plates were spotted (from left to right) with dansylated derivatives of the following standard polyamine solutions and perchloric acid cell extracts. (A) Spermidine (S) (200 pM); putrescine (P) (200 pM); cadaverine (C) (200 pM); JC182 extract; PSP01 extract; C. cadaverine (80 pM); putrescine (80 pM); spermidine (80 pM). (B) Spermidine (200 pM); putrescine (200 pM); cadaverine (200 pM); JC182 extract (4 × 10^7 cells); PSP01 extract (4 × 10^7 cells); PSP01 extract (2 × 10^7 cells); JC182 extract (2 × 10^7 cells); cadaverine (100 pM); putrescine (100 pM); spermidine (100 pM).*

slow residual growth without polyamine supplementation. The addition of putrescine or spermidine to the medium increases the growth rate of these strains to wild-type levels. When a polyamine auxotroph is grown under conditions of polyamine starvation, cadaverine is present in the perchloric acid-extractable pool (10). Presumably, cadaverine and its aminopropyl derivative are able to substitute partially for the growth-promoting properties of putrescine and spermidine. We measured the lysine decarboxylase level in MA255, a double polyamine auxotroph mutated in the genes for arginine ureohydrolase and ornithine decarboxylase (8), and found it to be the same as that of the wild-type parent. Thus, we concluded that cadaverine synthesis in polyamine-starved polyamine auxotrophs must occur via release of feedback inhibition of lysine decarboxylase rather than by induction of this enzyme. To test this further, matings to transfer the *lysP* marker of PSP01 into MA255 were performed. The resulting strain had a growth rate identical to that of its thiosine-sensitive parent, although its lysine decarboxylase level was derepressed more than 100 times in stationary cultures grown on minimal medium. This indicates that maximal cadaverine replacement of putrescine and spermidine takes place through release of feedback inhibition, since derepression of lysine decarboxylase, yielding additional enzyme, had no effect on the growth rate.

Other characteristics. The mutant PSP01 and its wild-type parent JC182 have the same doubling time in minimal medium, approximately 50 min. This contrasts with the thiosine-resistant mutants reported by Hirshfield and Zamecnik (18) and Jegede et al. (19), which are slow growing. We also assayed the following enzymatic activities and found them to be unaltered in the mutant: lysyl tRNA synthetase, inducible arginine decarboxylase, constitutive arginine decarboxylase, inducible ornithine decarboxylase, and constitutive ornithine decarboxylase. We tested the ability of the two strains (both lysine prototrophs) to feed three lysine auxotrophs blocked in diaminopimelic acid decarboxylase, the last step in lysine biosynthesis. Both the thiosine-resistant mutant and its parent excrete lysine. The feeding of lysine auxotrophs was observed with shorter incubation times when the mutant was the feeder and was apparent earlier in both strains when they were grown on the amino acid-enriched medium.

Mapping of the PSP01 mutation. Strain PSP01 is a derivative of the double-male strain JC182 (5). It was crossed with several multiply auxotrophic strains as recipients (MA140, 220Ag, and MA31). Recombinants were selected for prototrophy at eight loci distributed around the *E. coli* chromosome and scored for the inheritance of thiosine resistance. This marker was found to segregate in these crosses with *his*, but not with *pro*, *thr*, *leu*, *argG*, *argE*, *trp*, or *thy*. (Fig. 4.)

Since there are a number of Hfr strains with points of origin near *his* (Fig. 4), it was possible to further define the locus of the thiosine resistance marker by determining which of these strains would transfer this marker in a short interrupted mating. A thiosine-resistant female was constructed by mating PSP01 with MA74 (Table 1) and selecting for *his*$^+$ recombinants. These were scored for thiosine resistance, and recombinant strain PSP100, which has the genotype *thi arg aroD lysP*, was mated with each of the Hfr strains shown in Fig. 4 until the *aroD* marker entered. These *aroD*$^+$ recombinants were scored for thiosine sensitivity. There were no thiosine-sensitive recombinants among the *aroD*$^+$ recombinants in crosses with KL96 and Hfr44 as donors; with KL16 as donor 20 of 88 *aroD*$^+$ recombinants were thiosine sensitive and with KL98 as donor 41 of 80 *aroD*$^+$ recombinants were thiosine sensitive. Thus, the *lysP* marker must be located between the points of origins of KL98 and KL96, that is, between the *dsd* and *his* markers.

A histidine-requiring thiosine-resistant F$^-$ strain was constructed by direct selection of the thiosine resistance marker from PSP01 into MA140; this strain, PSP121, which has the genotype *thi thr leu arg pro trp his thyA lysP*, was

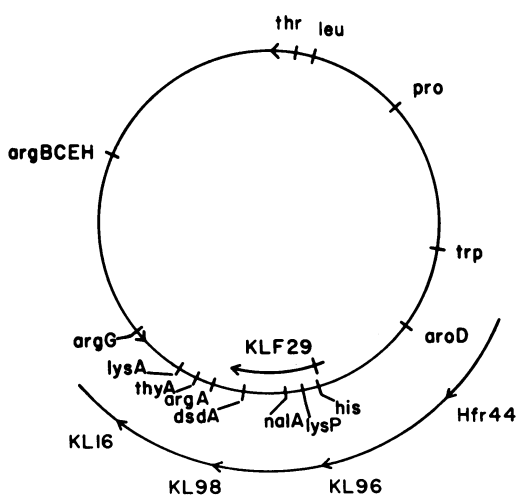

FIG. 4. *Linkage map of E. coli with pertinent markers. For references to marker genes, see Bachman et al. (1); for origins of Hfr strains, see Low (20). nalA = gyrA.*

used as the recipient in two mating experiments. In the first experiment PSP121 was mated with KL98; *his*$^+$ was selected for, and thiosine sensitivity was scored. Of 160 *his*$^+$ recombinants, 104 were found to be thiosine sensitive, indicating 65% linkage between these two markers in this cross. KL166, a *gyrA* derivative of KL16, was crossed with PSP121; *his*$^+$ was selected for, and both nalidixic acid resistance and thiosine resistance were scored. The distribution of recombinant classes is shown in Tables 5 and 6. The rare class of recombinants which were Nal^r and thiosine resistant indicated that the order of the three markers is *gyrA lysP his*. The linkage data confirm this order and indicate that *lysP* is about midway between the other two markers. These data are as follows: *his gyrA*, 54%; *his lysP*, 79%; *gyrA lysP*, 73%. It is interesting to note that this mutation does not map near the previously described basic amino acid decarboxylation and transport mutants, which are all clustered near and cotransducible with *serA* (22).

Matings were performed to determine the position of *metG* with respect to *his* and *lysP*. In a cross between KL98 and SB1803 (Table 1) there was 100% linkage of *metG* and *his* among *his*$^+$ recombinants. Mating of KL96 with SB1803 showed that *metG*$^+$ enters before *his*$^+$ with this donor. This establishes the following order: *gyrA lysP metG his*.

P1 bacteriophage grown on strain PSP01 was used to transduce *metG* and *his* strains. A total of 200 *metG*$^+$ transductants and 320 *his*$^+$ transductants were scored for thiosine resistance, but no cotransduction was found in either class of transductants. Transductions for *gyrA* were repeatedly attempted without success. Transduction for thiosine resistance was not feasible because of the high level of spontaneous mutation.

Analysis of thiosine resistance recombinants. Thiosine-resistant recombinants of the crosses described above and their female thiosine-sensitive parents were assayed for lysine transport and lysine decarboxylase activity with the following results: (i) the parent strains MA140 and MA74 have lower wild-type lysine transport ability than JC182 and 220Ag; (ii) all thiosine-resistant recombinants have lowered lysine transport compared with their parent strains, but the percentage of residual transport is higher in those strains which have a lower wild-type transport capability; (iii) the lysine decarboxylase levels of stationary cultures of thiosine-resistant recombinants between PSP01 and either of the strains with low wild-type lysine transport ability vary from wild-type level to the fully derepressed level of the mutant. The cause of the last finding is not known. The range of lysine decarboxylase levels seen in these recombinants indicated that several genes influencing the expression of the lysine decarboxylase gene might be segregating in these crosses. An isogenic recipient was therefore prepared for a study of the segregation of the PSP01 mutation and its effect on the expression of the lysine decarboxylase gene in an isogenic cross. This recipient, a *his rpsL* derivative of JC182, was converted to phenocopy and mated with PSP01 with selection for *his*$^+$ entry; 58 of 104 *his*$^+$ recombinants scored were thiosine resistant. Assays of the lysine transport and lysine decarboxylase levels of 13 *his*$^+$ recombinants showed no recombinants with intermediate levels of lysine decarboxylase. The four thiosine-sensitive recombinants tested resembled their female parent in both lysine uptake and lysine decarboxylase level; the nine thiosine-resistant recombinants all demonstrated both the lowered lysine transport and elevated lysine decarboxylase of the original PSP01 parent.

The fact that recombinants without elevated lysine decarboxylase are thiosine resistant indicates that the lowered lysine transport is the primary cause of resistance to thiosine, its analog. This view is supported by the fact that all of the independently isolated thiosine-resistant mutants of JC182 assayed in this laboratory showed lowered lysine uptake.

TABLE 5. *Recombinants of KL166 gyrA his*$^+$ *lysP*$^+$ × *PSP121 gyrA*$^+$ *his lysP*

Phenotype	Genotype	No.
Nal^r T^s	*gyrA lysP*$^+$	105
Nal^r T^r	*gyrA lysP*	3
Nal^s T^s	*gyrA*$^+$ *lysP*$^+$	52
Nal^s T^r	*gyrA*$^+$ *lysP*	40

TABLE 6. *Number of crossovers necessary to produce observed recombinant classes in Table 5*

Recombinant genotype	Possible parental gene orders		
	gyrA his$^+$ *lysP*$^+$ *gyrA*$^+$ *his lysP*	*gyrA lysP*$^+$ *his*$^+$ *gyrA*$^+$ *lysP his*	*lysP*$^+$ *gyrA his*$^+$ *lysP gyrA*$^+$ *his*
gyrA lysP$^+$	2	2	2
gyrA lysP	2	4	2
gyrA$^+$ *lysP*$^+$	2	2	4
gyrA$^+$ *lysP*	2	2	2

DISCUSSION

We have presented data characterizing a mutation with the following features. It confers resistance to a lysine analog (thiosine), lowered lysine transport by both the lysine-specific and LAO systems, elevated levels of lysine decarboxylase, lower sensitivity of lysine transport to

repression during growth in the presence of lysine, and more rapid excretion of endogenously synthesized lysine. The partially elevated lysine decarboxylase level seen in exponential cultures of this mutant does not lead to the accumulation of a measurable cadaverine pool, although the fully derepressed levels of the enzyme seen in stationary cultures of this strain do.

This mutation differs from previously described thiosine resistance mutations (7, 14, 15, 18, 19, 26, 29). The spontaneous origin and high frequency of isolation of thiosine-resistant mutants with the same properties as PSP01 indicate that the multiple phenotypic manifestations of this mutant are due to a single mutation. We cannot rule out the possibility that this mutation is a deletion spanning several adjacent loci. A deletion or polar nonsense mutation affecting the structural genes for components of the two lysine transport systems and the repressor for lysine decarboxylase might result in a similar phenotype. This partial derepression of lysine decarboxylase in exponential minimal cultures indicates that a functional repressor of lysine decarboxylase synthesis exists in the mutant and probably has altered affinity for either lysine, the effector, or the lysine decarboxylase operator locus. The *lysP* mutation segregates in isogenic matings as would be expected for a single mutation. However, in nonisogenic matings the expression of the alteration in lysine decarboxylase level is quite variable. Since this effect is not seen in isogenic crosses, we conclude that it is due to secondary effects of segregating modifying genes in nonisogenic crosses. The induction of lysine decarboxylase is known to be affected by pH and the presence of its substrate, lysine. Presumably, both lysine and hydrogen ions react with the lysine decarboxylase repressor, causing allosteric interactions which alter the affinity of this regulatory protein for the lysine decarboxylase operator. Genes that affect intracellular hydrogen ion or lysine concentration could modulate the effect of this mutation by altering the percentage of aporepressor in active form.

The phenotypic manifestations of the *lysP* mutation are most simply explained by a regulatory mutation affecting the expression of the lysine transport and lysine decarboxylase genes. Both of these functions are regulated by lysine. The lysine transport systems are repressible by lysine, whereas lysine decarboxylase is inducible by lysine. We propose that a single regulatory protein could regulate both of these functions if when bound to lysine, it was a functional repressor of the transport genes and when free of lysine, it was a functional repressor of the decarboxylase gene. If such a regulatory protein were altered by mutation to increase its affinity for lysine, one would see the effects on lysine transport and lysine decarboxylase activities manifested by the mutant PSP01. Thus, we propose that the *lysP*$^+$ locus codes for a common regulatory protein that mediates the repression of the lysine-specific and LAO transport systems and lysine decarboxylase. The *lysP* mutation may be analogous to the mutations in the *lac* system which show altered affinity for the entire spectrum of *lac* operon inducers (2, 3).

The *lysP* mutation was found to map between the *gyrA* and *metG* markers on the *E. coli* chromosome. There was no cotransduction of *lysP* with *metG* or the adjacent *his* marker. Attempts were made to study the behavior of the *lysP* mutation in diploids by using KLF29 (Fig. 4) and similar F′ strains isolated in this laboratory from KL98 which span the *dsd-his* segment of the *E. coli* chromosome. The resulting diploid strains repeatedly showed the characteristics determined by the endogenote marker, regardless of whether the wild-type or mutant allele was in this location. This result led us to conclude that these F′ strains must be unstable for the genes being considered and therefore not suitable for reliable dominance studies at the present time.

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