JOURNAL OF BACTERIOLOGY, Mar. 1991, p. 1965–1970
0021-9193/91/061965-06$02.00/0

Vol. 173, No. 6

Partial Characterization of a *lysU* Mutant of *Escherichia coli* K-12

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The *Escherichia coli* K-12 strain GNB10181 shows no inducible lysyl-tRNA synthetase (LysRS) activity. Two-dimensional gel electrophoretic analysis of the polypeptides synthesized by this strain indicates that the normal *lysU* gene product, LysU, is absent. When both GNB10181 and its parent, MC4100, were grown at elevated temperatures (42 to 45°C) no significant difference between their growth rates was observed. The *lysU* mutation was transferred to other *E. coli* K-12 backgrounds by using P1 transduction. The *lysU* transductants behaved comparably to their *lysU*$^+$ parents at different growth temperatures. Therefore, the LysU protein does not appear to be essential for growth at high temperatures, at least under the conditions examined here. In addition, *lysU* transductants were found to be defective for inducible lysine decarboxylase, (LDC), inducible arginine decarboxylase (ADI), and melibiose utilization (Mel), which are all missing in GNB10181. Complementation of the above missing functions was achieved by using the Clarke-Carbon plasmids pLC4-5 (LysU LDC) and pLC17-38 (LysU Mel ADI). From these experiments, it appears that GNB10181 has suffered a chromosomal deletion between 93.4 and 93.7 min, which includes the *lysU* gene. By using plasmid pLC17-38, the position of ADI on two-dimensional gels was identified. Finally, *lysS* Δ*lysU* double mutants were constructed which can potentially be used as positive selection agents for the isolation of LysRS genes from other sources.

All cells which have so far been examined preferentially synthesize a family of proteins, known as heat shock proteins, when exposed to elevated temperatures (1). In *Escherichia coli*, more than 17 heat shock proteins are known, some of which have been identified (22). One of these proteins, LysU, is the product of an inducible form of lysyl-tRNA synthetase (LysRS), which has been designated D60.5 on the basis of its position on two-dimensional (2-D) gels (14, 16). The LysU protein is normally not expressed at a significant level but can be induced by a number of conditions, including heat shock (13, 14, 18).

LysRS in *E. coli* K-12 is unique in that it is encoded by two differentially regulated genes (14, 16). The constitutive gene, *lysS*, maps at 62.1 min (9), whereas the inducible gene, *lysU*, has been localized to 93.5 min (28). *lysS* mutants and pseudorevertants derived from them, which constitutively express *lysU* because of an *rlu* (for regulation of *lysU*) mutation, have been isolated previously (16). A *lysU* mutant would be useful in determining whether the heat-inducible enzyme LysU is essential for the growth of *E. coli* at elevated temperatures.

In this study, we report the partial characterization of an *E. coli* strain that shows no inducible LysRS activity. 2-D gel analysis indicates that this strain does not express the normal *lysU* gene products. From the results of complementation experiments, it appears that the lack of *lysU* expression in this mutant is due to a chromosomal deletion, which extends at least from 93.4 to 93.7 min, including the genes for inducible lysine decarboxylase (*cadA*), inducible LysRS (*lysU*), melibiose utilization (*mel* operon), and inducible arginine decarboxylase (*adi*). Data are presented demonstrating that the heat-inducible LysRS, LysU, is not essential for the growth of *E. coli* at high temperature. We also report the construction of *lysS* Δ*lysU* double mutants which can be used to isolate LysRS genes from other sources.

(A preliminary account of this work was presented at the 1990 Annual Meeting of the American Society for Microbiology [12a].)

MATERIALS AND METHODS

Bacterial strains. All bacterial strains used in this study are derivatives of *E. coli* K-12 and are listed in Table 1. The strain OEL134 has a *lysS* mutation, which causes a K_m deficiency for lysine. As a result, this strain grows poorly in the absence of lysine but normally in 0.12 mM lysine (4). MH51 is a pseudorevertant of OEL134 that expresses *lysU* constitutively and therefore can grow normally without lysine. The latter phenotype has been shown to be the result of an *rlu* mutation which has been described previously (16).

Media and culture conditions. Cells were initially grown overnight in minimal medium (8) supplemented with amino acids (including lysine only when it was necessary), vitamins, and bases (SMM [23]), with glucose (0.036% [vol/vol]) as a carbon and energy source, in a New Brunswick Scientific Controlled Environment Incubator Shaker at 125 rpm at either 30 or 37°C. The overnight cultures were then diluted into fresh SMM containing 0.36% (vol/vol) glucose and subsequently shaken at 200 rpm. The SMM cultures were used to assess the growth rates of the strains or the inducibility of LysRS by 3 mM glycyl-L-leucine (Sigma Chemical Co.) at 37°C. In experiments in which strains were tested for growth at elevated temperatures (between 42 and 45°C), the overnight cultures (30°C) were diluted 1/200 and then allowed to grow at 30°C to an optical density of 0.005 to 0.015 at 580 nm (Perkin-Elmer Coleman 295 Spectrophotometer) before shifting to the higher temperature. Cells were also induced for LysRS by growth at 37°C in AC broth (Difco) to late log or early stationary phase until the pH of the culture declined to 5.5 or less (13). The Mel$^+$ phenotype was tested by growth on minimal or MacConkey agar base plates containing melibiose (1%).

P1 transduction and construction of *lysU* strains. A high-

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TABLE 1. Bacterial strains

Strain	Relevant properties	Source or reference
MC4100	*lysS*$^+$ *adi*$^+$ *mel*$^+$ *lysU*$^+$ *cadA*$^+$	B. J. Bachmann
GNB10181	*lysS*$^+$ Δ(*adi mel lysU cadA*) Ap^r Clr^r	G. N. Bennett (2)
AT2092	*lysS*$^+$ *adi*$^+$ *mel*$^+$ *lysU*$^+$ *cadA*$^+$	Lab collection (9)
HfrH	*lysS*$^+$ *adi*$^+$ *mel*$^+$ *lysU*$^+$*cadA*$^+$	B. J. Bachmann
OEL134	*lysS*(K_m^{Lys}) *lysU*$^+$	Boy et al. (4)
MH51	*rlu lysS*(K_m^{Lys})	Spontaneous revertant of OEL134 (this work)
MH108	*lysS*$^+$ Δ(*adi mel lysU cadA*) Ap^r Clr^r	P1 *vir* transductant of HfrH (this work)
MH205	*lysS*$^+$ Δ(*adi mel lysU cadA*) Ap^r Clr^r	P1 *vir* transductant of AT2092 (this work)
MH318	*lysS*(K_m^{Lys}) Δ(*adi mel lysU cadA*) Ap^r Clr^r F$^-$	P1 *vir* transductant of OEL134 (this work)
MH505	*rlu lysS*(K_m^{Lys}) Δ(*adi mel lysU cadA*) Ap^r Clr^r F$^-$	P1 *vir* transductant of MH51 (this work)
JA200/pLC4-5	F$^+$ (*lysU*$^+$ *cadA*$^+$)	B. J. Bachmann
JA200/pLC17-38	F$^+$ (*adi*$^+$ *mel*$^+$ *lysU*$^+$)	B. J. Bachmann

titer P1 *vir* bacteriophage lysate of GNB10181 was prepared and transductions were carried out as described by Silhavy et al. (26). The four recipient strains included two *lysS*$^+$ *lysU*$^+$ strains (i.e., AT2092 and HfrH), a *lysS*(K_m^{Lys}) *lysU*$^+$ strain (i.e., OEL134), and a *lysS*(K_m^{Lys}) *rlu* strain (i.e., MH51). To isolate *lysU* transductants of these recipients, Clr^r Amp^r transductants were selected on LB plates, supplied with either chloramphenicol (20 μg/ml) or ampicillin (45 μg/ml) and with lysine (0.12 mM) when necessary (i.e., for OEL134 and MH51), and screened for deficiencies in inducible lysine decarboxylase (LDC) and melibiose utilization (Mel). Strains deficient in these phenotypes were then tested for inducible LysRS.

Transformation. GNB10181 was made competent by the method of Hanahan (12) and transformed with 50 ng of pLC17-38 plasmid DNA. Transformants were selected on LB plates supplemented with colicin (5 μl/ml).

Conjugation. The F$^+$ strain JA200(pLC4-5) was used as the donor and MH318 and MH505 (Table 1) were used as the recipients in these experiments. Conjugation was done essentially as described by Miller (20). To achieve F-mediated transfer of plasmid pLC4-5, the matings were done for 2.5 h. Mating mixtures were washed twice with SMM containing no lysine. Transconjugants were selected on SMM plates without lysine and containing chloramphenicol (20 μg/ml). Both the donor and recipients were unable to grow on these plates.

Plasmid extraction. Plasmids were isolated from donors, transformants, and transconjugants by using the Pharmacia plasmid miniprep kit according to the specifications of the manufacturer and visualized by agarose gel electrophoresis with 0.5 μg of ethidium bromide per ml (26).

Enzyme assays. LysRS activity in whole-cell extracts was determined by the standard assay conditions described elsewhere (15). LDC was examined by using LDC Broth (Difco), and inducible arginine decarboxylase was examined by using Decarboxylase Base Broth (Difco) supplemented with arginine.

2-D gel electrophoresis of polypeptides. Cell extracts were prepared as described previously (13). Approximately 15 μg of total cellular protein, as determined by the method of Lowry (19), was loaded on each gel and resolved by 2-D gel electrophoresis essentially as described by O'Farrell (24). Silver staining was done by the method of Wray et al. (29).

RESULTS

Absence of inducible lysyl-tRNA synthetase activity in GNB10181. GNB10181, an *E. coli* strain derived from MC4100 by Mu*dlac* fusion (2), was grown under conditions that are known to induce LysRS activity (13, 14, 18). These conditions included growth in the presence of the peptide glycyl-L-leucine (3 mM) and growth in AC broth (a rich medium) to pH 5.3. In both cases, normal induction of LysRS activity (approximately two- to threefold) was observed in MC4100 but not GNB10181 (Table 2).

Absence of normal *lysU* gene products from GNB10181. The normal *lysU* gene products appear as a major (LysRS form II) and a minor (LysRS form IV) polypeptide on 2-D gels of *E. coli*, with similar apparent molecular masses (ca. 60.5 kDa) but slightly different isoelectric points (14). Figure 1 shows silver-stained 2-D gels prepared from MC4100 and GNB10181. The LysU protein can be observed in the gel prepared from MC4100 (Fig. 1B) but not in that prepared from GNB10181 (Fig. 1D) under the induced conditions. Similar results were obtained when these two strains were grown with 3 mM glycyl-L-leucine. It appears, therefore, that the normal LysU is not synthesized in GNB10181.

The *lysU* mutant is not temperature sensitive. When GNB10181 and MC4100 were grown at elevated temperatures (from 42 and 45°C) no significant differences between their growth rates were observed. Table 3 shows the exponential growth rates of both strains at different growth temperatures.

Effect of the *lysU* mutation in other *E. coli* K-12 backgrounds. To demonstrate that the growth behavior of GNB10181 is not unique to its particular genetic background, the *lysU* mutation was transferred from this mutant to two other *E. coli* K-12 strains, HfrH and AT2092, by P1 transduction. The Clr^r and Amp^r determinants in GNB10181

TABLE 2. Lysyl-tRNA synthetase activity of Δ*lysU* strains and their *lysU*$^+$ parents

Strain	Activity (U/mg of protein)	
	Uninduced[a]	Induced
MC4100	72.0	167.0[b]
	63.0	187.0[c]
GNB10181	51.0	50.0[b]
	57.4	58.4[c]
HfrH	107.0	224.0[c]
MH108	114.0	143.0[c]
AT2092	72.0	120.0[b]
MH205	65.5	59.5[b]

[a] LysRS activity in SMM.
[b] LysRS activity in SMM containing the inducer glycyl-L-leucine (3 mM).
[c] LysRS activity in AC broth (pH 5.3).

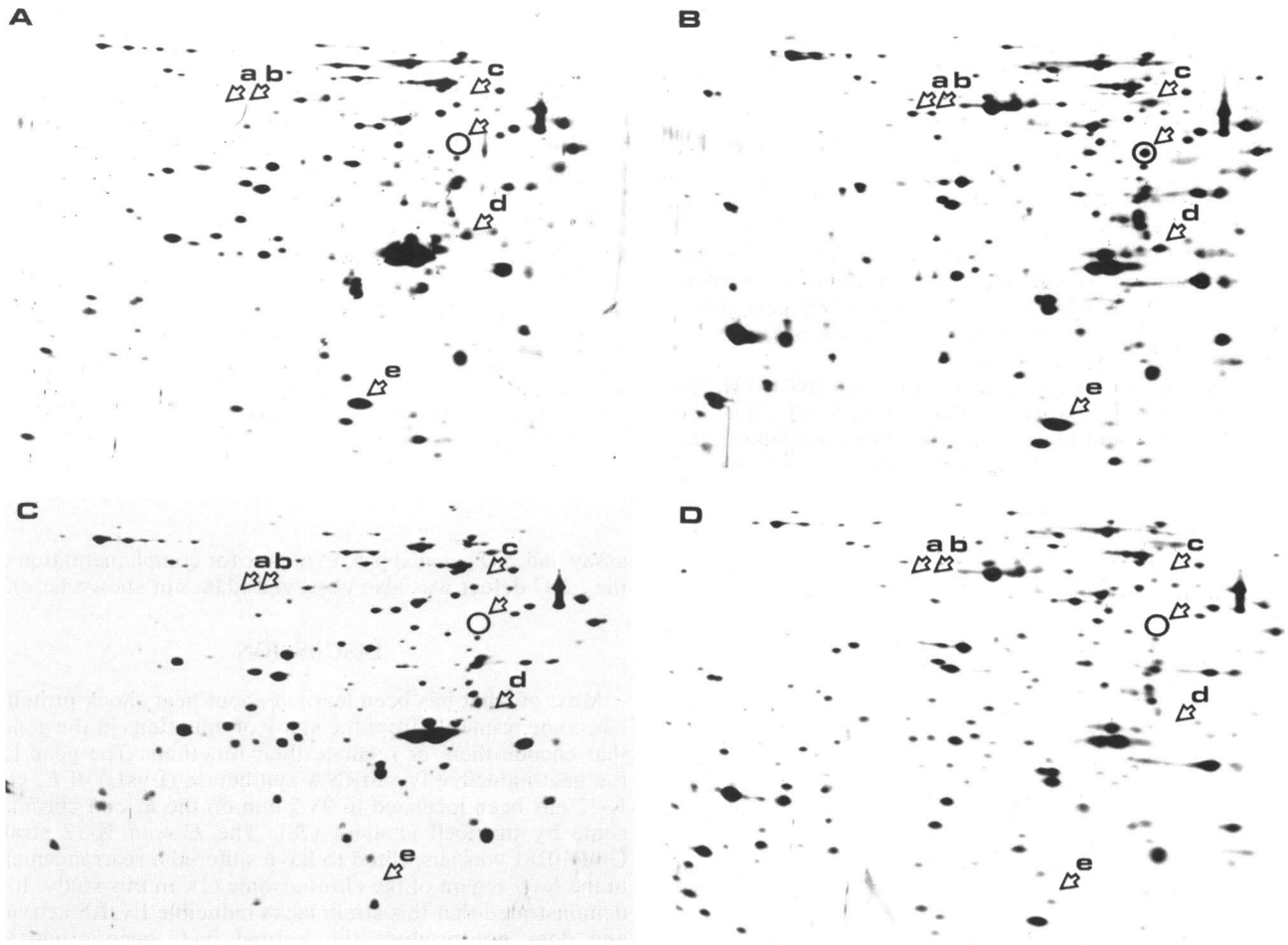

FIG. 1. Silver-stained 2-D gels prepared from MC4100 grown in SMM (A), MC4100 grown in AC broth to pH 5.3 (B), GNB10181 grown in SMM (C), and GNB10181 grown in AC broth to pH 5.3 (D). Whole-cell extracts (15 μg) were loaded on gels. Open arrows point to the position of altered or missing polypeptides in GNB10181. ○, Position of the inducible lysyl-tRNA synthetase (LysU). a and b represent the inducible lysine decarboxylase (LDC), and c represents the inducible arginine decarboxylase (ADI). d and e were not identified. More-acidic polypeptides are located on the right side of each gel.

(from Mu*dlac* [2]) were suspected to have been retained at or near the site of a chromosomal rearrangement that resulted in the *lysU* genotype in this strain. Therefore, several Clr^r Amp^r transductants of HfrH and AT2092 were examined for inducible LysRS activity. Table 2 shows the results of LysRS assays on two transductants, MH108 and MH205, and their *lysU*$^+$ parental strains, HfrH and AT2092, respectively. Both transductants failed to show normal LysRS induction. 2-D gel analysis confirmed that MH108 and MH205 do not synthesize LysU (data not shown here). Furthermore, the *lysU* transductants, MH108 and MH205, were also found to be lacking other functions, including LDC, ADI, and Mel, which have been reported to be missing in GNB10181 (2; see below). Finally, when MH108, MH205, and their *lysU*$^+$ parental strains were grown at elevated temperatures, the mutants grew at rates comparable to those of their parents (Table 3).

TABLE 3. Generation times of the Δ*lysU* strains and their *lysU*$^+$ parents at different growth temperatures

Strain	Generation time (min)[a] at:			
	30°C	37°C	43.5°C	45°C
MC4100 (parent)	31.5	29.5	42.5	70.0[b]
GNB10181 (mutant)	32.5	25.5	39.5	65.0[b]
AT2092 (parent)	43.0	26.5	32.5	50.0
MH205 (transductant)	44.0	27.0	34.0	51.5
HfrH (parent)	37.5	34.0	(29.0)[c]	—
MH108 (transductant)	37.5	32.5	(30.0)[c]	—

[a] Generation times were determined from the mid-log phase of the growth curves and represent the averages of duplicate trials.
[b] In this experiment twice the concentration of basal medium was used.
[c] In this experiment cultures were grown at 42°C, since HfrH and its derivative, MH108, do not grow well above this temperature.

Other missing and altered gene products in GNB10181. The genes coding for LDC (*cadA*), ADI (*adi*), and Mel (*mel* operon) have been shown to map near the *lysU* gene on the *E. coli* chromosome (2, 3; Fig. 2). 2-D gel analysis of the polypeptides of GNB10181 and MC4100 showed a number of differences in addition to LysU (Figure 1). Polypeptides a, b, and c are missing in GNB10181, whereas polypeptides d and e appear to be either missing or significantly altered in their amounts in this strain. The apparent molecular weights and isoelectric points of polypeptides a and b correspond to

those for LDC, according to the gene-protein index of *E. coli* (25). We have identified polypeptide c as the ADI of *E. coli* K-12 on the basis of the following evidence. (i) This polypeptide appears to be induced in AC broth under conditions which are known to be optimum for induction of ADI (i.e., low pH and semianaerobic growth [2, 10, 21]). (ii) Polypeptide c is present in *adi*$^+$ strains and is missing in *adi* mutant strains. (iii) Both the lack of ADI activity and the absence of polypeptide c in GNB10181 were complemented by plasmid pLC17-38, which carries *adi* (see below). (iv) Finally, the apparent molecular weight and isoelectric point of a purified *E. coli* arginine decarboxylase (Sigma) on 2-D gels correspond to those for polypeptide c. The other two polypeptides (d and e in Fig. 1) were not identified.

Complementation of the *lysU* mutation of GNB10181. To further demonstrate that GNB10181 has suffered a deletion of the *lysU* gene and not a regulatory mutation influencing the expression of this gene, this mutation was complemented by two plasmids from the Clarke-Carbon collection (7). Plasmid pLC17-38 has been shown to express the genes necessary for melibiose utilization (27) and has been suggested to express *lysU* (3, 11). This plasmid was transformed into GNB10181. A *mel*$^+$ transformant was examined by the standard LysRS assay and showed inducible LysRS activity (Table 4). To verify this transfer, the plasmid was reisolated and visualized by agarose gel electrophoresis (data not shown). In addition, 2-D gel analysis showed that both LysU and ADI (polypeptide c in Fig. 1) are synthesized in the transformant (Fig. 3). These results represent the first direct demonstration that plasmid pLC17-38 expresses LysU. The presence of *adi* on plasmid pLC17-38 correlates with the recent mapping of this gene at 93.4 min (2).

To enhance our selection for another *lysU*-encoding plasmid, pLC4-5 (28), *lysS* Δ*lysU* double mutants MH318 and MH505 were constructed by P1 transduction. These constructs are unable to grow in SMM in the absence of lysine, because of the K_m^{Lys} defect in their *lysS* gene products and the lack of LysU. pLC4-5 was transferred to the above-described recipients by F-mediated conjugation. The plasmid was reisolated from the transconjugants by plasmid miniprep and visualized by agarose gel electrophoresis (data not shown). Transconjugants that were able to grow on lysine-deficient media were shown, by enzyme assays (Table 4) and 2-D gel analysis (data not shown), to express the *lysU* gene, even in the absence of an inducer. Furthermore, it was demonstrated that upon bringing the *lysU* gene into an *rlu* background (MH505), the ability to constitutively express *lysU* was retained (Table 4). Finally, by using the LDC broth assay and 2-D gel analysis, evidence for complementation of the LDC defect was also observed (data not shown here).

TABLE 4. Complementation of the inducible lysyl-tRNA synthetase activity of Δ*lysU* and *lysS* Δ*lysU* mutants by *lysU*-encoding plasmids

Strain	Activity (U/mg of protein)	
	Uninduced	Induced[a]
GNB10181	52.1	52.9
GNB10181/pLC17-38	57.8[b]	135.9[b]
GNB10181/pLC17-38	57.3	190.7
OEL134	1.15	68.7
MH318	0.7	0.8
MH318/pLC4-5	16.5	559.8
MH51	57.2	148.2
MH505	ND[c]	ND
MH505/pLC4-5	500.5	612.7

[a] LysRS induction was achieved by growth in AC broth to pH 5.3.
[b] Melibiose (0.36%) was used as a carbon and energy source, and LysRS induction was achieved by glycyl-L-leucine (3 mM).
[c] ND, Not detected.

DISCUSSION

Most of what has been learned about heat shock proteins has come primarily from the study of mutations in the genes that encode them or regulate their functions. The gene for the heat-inducible lysyl-tRNA synthetase (LysU) of *E. coli* K-12 has been localized to 93.5 min on the *E. coli* chromosome by maxicell analysis (28). The *E. coli* K-12 strain GNB10181 was suspected to have suffered a rearrangement in the *lysU* region of the chromosome (2). In this study, it is demonstrated that this strain lacks inducible LysRS activity and does not produce the normal *lysU* gene products. Furthermore, evidence from data presented here suggests that the absence of inducible LysRS in this mutant is not due to a regulatory effect. Both superrepression and the absence of a functional positive regulator in the Δ*lysU* mutants GNB10181, MH318 and MH505 appear as unlikely events since the inducible expression of *lysU* in GNB10181 and MH318 and the constitutive expression of *lysU* in MH505

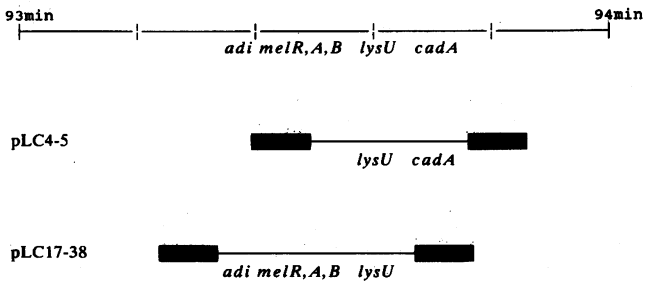

FIG. 2. Position of relevant genes on the *E. coli* chromosome (top) and ColE1 plasmids pLC4-5 (middle) and pLC17-38 (bottom) (2, 3, 27, 28, and this work). The line represents *E. coli* chromosomal DNA, and the solid bar represents ColE1 plasmid DNA.

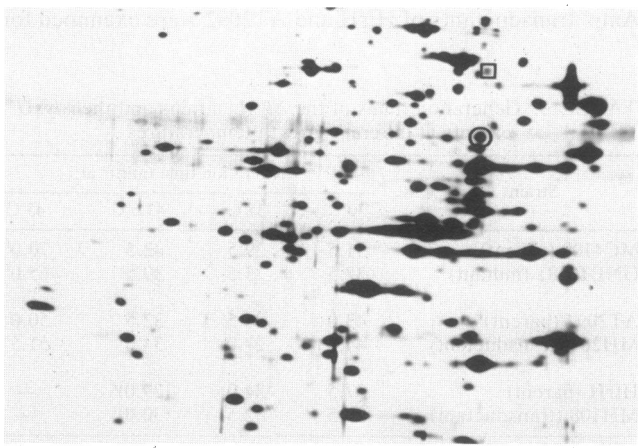

FIG. 3. Silver-stained 2-D gel prepared from a GNB10181/pLC17-38 transformant grown in AC broth to pH 5.3. Twenty micrograms of the whole-cell extract was loaded on the gel. The positions of LysU (○) and ADI (□) are shown.

(i.e., an *rlu* background) were regained upon introducing a *lysU*-encoding plasmid into these strains. In addition, the results of the complementation experiments confirm the suggestion that GNB10181 carries a relatively large chromosomal deletion between 93.4 and 93.7 min (2) that includes *cadA*, *lysU*, the *mel* operon, *adi*, and most probably a number of other genes, including *fumB*, *pheR*, and *bymA*, which have been mapped to this region of the *E. coli* chromosome (3, 11).

According to our growth studies, none of the missing functions in GNB10181 appear to be absolutely essential for normal growth behavior, at least in the media and under the conditions used in this study. More specifically, neither GNB10181 nor any of the Δ*lysU* transductants examined in this study (MH108 and MH205) behave differently at elevated growth temperatures, when compared with their *lysU*$^+$ parental strains. These results immediately point out the intriguing observation that the *lysS* gene product appears to be sufficient for supporting the growth of a Δ*lysU* mutant even at elevated temperatures. Furthermore, although previous in vitro work suggests that LysS is a heat-labile enzyme (17), it appears that growth of Δ*lysU* strains at elevated temperatures, as examined in this study, does not lead to an irreversible loss of LysS activity (data not shown).

Our results seem to be in contrast with those in a very recent publication, by Clark and Neidhardt (6), in which a Δ*lysU* strain was reported to have a growth defect at high temperature (44°C) in a rich medium. It should be emphasized that in our experiments we employed three strains, with different genetic backgrounds, two of which were grown at 45°C without showing a growth defect. Moreover, in these growth experiments the Δ*lysU* mutant cultures were shifted to elevated temperatures at a very low density to permit adequate time for a growth defect to manifest itself.

To continue the investigation of the role of LysU in *E. coli*, we exposed GNB10181 and its *lysU*$^+$ parent to heat shock at 50°C and studied their survival over a 2-h time period after the temperature upshift. In our preliminary studies we did not observe any significant difference between the ability of these two strains to survive this heat stress (data not shown). In addition, no differences in the major heat shock proteins, DnaK and GroEL, were detectable on 2-D gels prepared from the two strains after heat shock (data not shown).

Our growth data and heat shock survival experiments are not consistent with an important role for the LysU protein at high temperature. An alternative role for this protein has been suggested in a recent report from this laboratory, which has shown that LysU is well induced when cells are grown in a rich medium (AC broth) to a low pH (13). On the basis of standard enzyme assays and 2-D gel analysis we believe the latter physiological condition to be the optimum for induction of LysU in *E. coli*. It is evident, therefore, that in searching for a raison d'être for the LysU protein, its potential role in the low-pH response of *E. coli* in rich medium should be considered. It is interesting that C62.5 (*htpG* gene product), another heat shock protein of *E. coli* K-12, is also induced under similar conditions (i.e., in AC broth at low pH [13]). Furthermore, experiments done with an *htpG* mutant have demonstrated that C62.5 is also not essential for growth at elevated temperatures, although this mutant showed a slight growth disadvantage at higher temperatures (5). The fact that *lysU*, *cadA*, and *adi* are all located in the same region of the *E. coli* chromosome may be of significance. These genes are apparently not close enough to form an operon, but they probably constitute at least part of a regulon. Preliminary experiments indicate that LysU and LDC can be coordinately induced at low pH in broth (13a). The assignment of the position of the *adi* gene product of *E. coli* on 2-D gels increases the feasibility of analyzing the potential coregulation of the inducible arginine decarboxylase, inducible lysine decarboxylase, and inducible lysyl-tRNA synthetase in *E. coli*.

Finally, *lysS* Δ*lysU* strains constructed here should prove useful as positive selection agents in the isolation of LysRS genes from other sources, as long as they can be expressed in *E. coli*.

ACKNOWLEDGMENTS

We thank B. J. Bachmann for sending us the strains used in this study.

We thank St. John's University for supporting this study. This work was additionally supported by a grant-in-aid of research from Sigma Xi.

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