APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1992, p. 2704–2707
0099-2240/92/082704-04$02.00/0

Vol. 58, No. 8

Temperature-Dependent Induction of an Acid-Inducible Stimulon of *Escherichia coli* in Broth

MORAD HASSANI,[1] DAVID H. PINCUS,[1] GEORGE N. BENNETT,[2] AND IRVIN N. HIRSHFIELD[1]*

Department of Biological Sciences, St. John's University, Jamaica, New York 11439,[1] and Department of Biochemistry, Rice University, Houston, Texas 77251[2]

Received 2 December 1991/Accepted 8 May 1992

The induction of the inducible lysyl-tRNA synthetase, LysU, and the inducible lysine and arginine decarboxylases of *Escherichia coli* K-12 grown in AC broth to a pH of 5.5 or less is temperature dependent, being distinctly lower at 24 than at 37°C. This induction does not appear to be under HtpR control.

Recently, there has been recognition of the need to examine the molecular response of bacteria to acidic pH conditions (3, 4, 11, 12, 27). There is ample motivation to study this response, since acidic pH is commonly a natural barrier to bacterial growth. Nevertheless, bacteria can habituate to low pH conditions (4, 7, 26), and as a consequence, they may be more dangerous as pathogens when ingested by humans or mammals (8, 26). These considerations underscore the need to identify the genes and their products which enable microorganisms to adapt to low pH conditions.

The lysyl-tRNA synthetase (LysRS) system of *Escherichia coli* K-12 consists of two genes, *lysS*, which is constitutive, and an inducible gene, *lysU* (13, 15). The *lysU* gene product, LysU, is induced by heat shock (13, 21) and growth in AC broth to an external pH (pH_o) of 5.5 or less, which is the best LysU-inducing condition (12). Classically, growth of *E. coli* in broth to a pH_o of 5.5 or less results in an increase in the activities of inducible lysine decarboxylase (LDC) and inducible arginine decarboxylase (ADI) (6).

In this report, it is demonstrated that LysU, LDC, and ADI, as well as the heat-shock polypeptides HtpG and HtpM (21, 22), are molecular components of a stimulon that is induced in a temperature-dependent manner when *E. coli* is grown in broth to low pH_o.

The *E. coli* K-12 prototrophs MG1655 and EMG2 and the isogenic pair SC122 (*htpR*$^+$) and K165 [*htpR1*(Am)] were obtained from the *E. coli* Genetic Stock Center, Yale University. The *htpR* gene has been redesignated *rpoH* and encodes the sigma-32 polypeptide (9). GNB7145, an *adi*::Mud*lac* fusion strain (*adi* encodes inducible ADI), and GNB8385, a *cadA*::Mud*lac* fusion strain (*cadA* encodes LDC), were derived from strain MC4100 Δ(*argF-lac*)*205 relA1 rpsL150* as described by Auger et al. (1).

Uninduced cultures were grown to mid-log phase (pH_o 6.7 to 6.8) in minimal medium (2), supplemented with amino acids, vitamins, and bases (SMM) (23), with 0.36% (vol/vol) glucose (10, 14). Induced cultures were grown in AC broth (Difco Laboratories, Detroit, Mich.). The AC broth cultures (initial pH_o, 6.90) were grown until the pH_o dropped to 5.2 to 5.3 (early stationary phase). In both media, the cells were grown with shaking at 200 rpm at appropriate temperatures. The pH_o was monitored by using an Orion Research model 611 digital pH meter.

Modified Falkow medium buffered with 100 mM MES (morpholineethanesulfonic acid) at pH 5.5 with or without 0.5% L-arginine or 0.5% L-lysine was made according to the procedure of Auger et al. (1). Strains were grown without shaking to late log phase at the appropriate temperature in screwcap tubes (16 by 125 mm) filled to the top (microaerobic condition). The uninduced controls for these experiments were grown as described above, conditions under which no induction of LysU or β-galactosidase via *cadA* or *adi* expression occurs. In all experiments, cell growth was monitored with a Perkin-Elmer 295 Coleman Spectrophotometer.

Lysyl-tRNA synthetase was assayed as described previously (14) with the following two modifications: ATP was used at a final concentration of 2 mM, and L-lysine was used at a final concentration of 10 μM. β-Galactosidase was assayed according to the method of Miller by using the chloroform and 0.1% sodium dodecyl sulfate option to permeabilize the cells (19). For two-dimensional (2-D) gel electrophoresis, cell extracts were prepared as described previously (12). Protein was determined by using the method of Lowry et al. (17). The 2-D gel electrophoretic procedure was done essentially as described by O'Farrell (24) with 15 μg of total protein applied per gel. The silver staining procedure used was that of Wray et al. (29). The positions of polypeptides on 2-D gels were determined from the gene-protein index (25) or independently (10).

Prior work has demonstrated that all factors which enhance LysRS activity in *E. coli* do so by elevating the amount of LysU, as detected by 2-D gel electrophoresis (12, 13, 15). Table 1 shows that there is a temperature-dependent increase in LysRS activity in *E. coli* K-12 strains MG1655 and EMG2 grown in AC broth to pH_o 5.2 to 5.3, with the largest increase occurring at 37°C. A corresponding temperature-dependent increase in the amount of LysU was detected on 2-D gels prepared from strain MG1655 (Fig. 1). Furthermore, we observed a temperature-dependent increase in the amounts of LDC and ADI and the heat-shock polypeptides HtpG and HtpM, as well as those of the three unidentified polypeptides c, d, and e. Essentially similar results were found with strain EMG2.

In order to further demonstrate the coregulation of *cadA* and *adi* with *lysU*, the *cadA*::*lac* and *adi*::*lac* fusion strains GNB8385 and GNB7145, respectively, were used. Initially, these strains were grown at 37°C in AC broth, and samples were taken at different pH_o points (i.e., pH_o 6.7, 6.0, 5.5, and 5.3), and examined for LysRS and β-galactosidase activities. It was found that LysRS and β-galactosidase activities were highest at the lowest pH_o point (early stationary phase)

* Corresponding author.

TABLE 1. Lysyl-tRNA synthetase activities of the prototrophic strains at different temperatures

Strain and temp (°C)	Act (U/mg of protein)		Induction ratio (induced/uninduced)
	Uninduced[a]	Induced[b]	
MG1655			
24	83.2	89.9	1.08
28	97.6	142.5	1.46
37	111.5	318.9	2.86
EMG2			
24	87.1	96.1	1.10
28	82.9	95.6	1.15
37	77.8	189.1	2.43

[a] LysRS activity in SMM at pH_o 6.7 to 6.8.
[b] LysRS activity in AC broth at pH_o 5.2 to 5.3.

examined (i.e., 6-fold induction of β-galactosidase activity in the *adi* strain, 30-fold induction of β-galactosidase activity in the *cadA* strain, and 2- to 3-fold induction of LysRS activity in both strains [data not presented]). In a second set of experiments, these strains were grown in AC broth at 24, 28, and 37°C to a pH_o of 5.2 to 5.3 and were examined for LysRS and β-galactosidase activities (Table 2). Both activities are highest at 37 and lowest at 24°C. As a control, β-galactosidase was induced with IPTG (isopropyl-β-D-thiogalactopyranoside) and measured in MG1655 grown in AC broth to low pH_o at 24 and 37°C. The activity was only 1.6-fold higher at 37°C.

E. coli GNB7145 and GNB8385 were also grown in modified Falkow medium (pH_o 5.5) at 24 and 37°C and assayed for both LysRS and β-galactosidase activities. These experiments yielded results similar to those found with cells grown in AC broth with respect to temperature-dependent behavior (Table 3). The experiments with the *adi* fusion strain GNB7145 showed that there was a three- to fivefold higher β-galactosidase activity at 37 than at 24°C, whether or not 0.5% L-arginine was present in the culture medium. However, the presence of 0.5% L-lysine in the culture medium resulted in a marked increase in the production of β-galactosidase in the *cadA* fusion (GNB8385) strain at 24°C, which clearly reduced the degree of temperature dependence of *cadA* expression observed. LysRS activity in the *cadA*::*lac* fusion strain also displayed temperature-dependent behavior. It was observed that, at 24°C under this

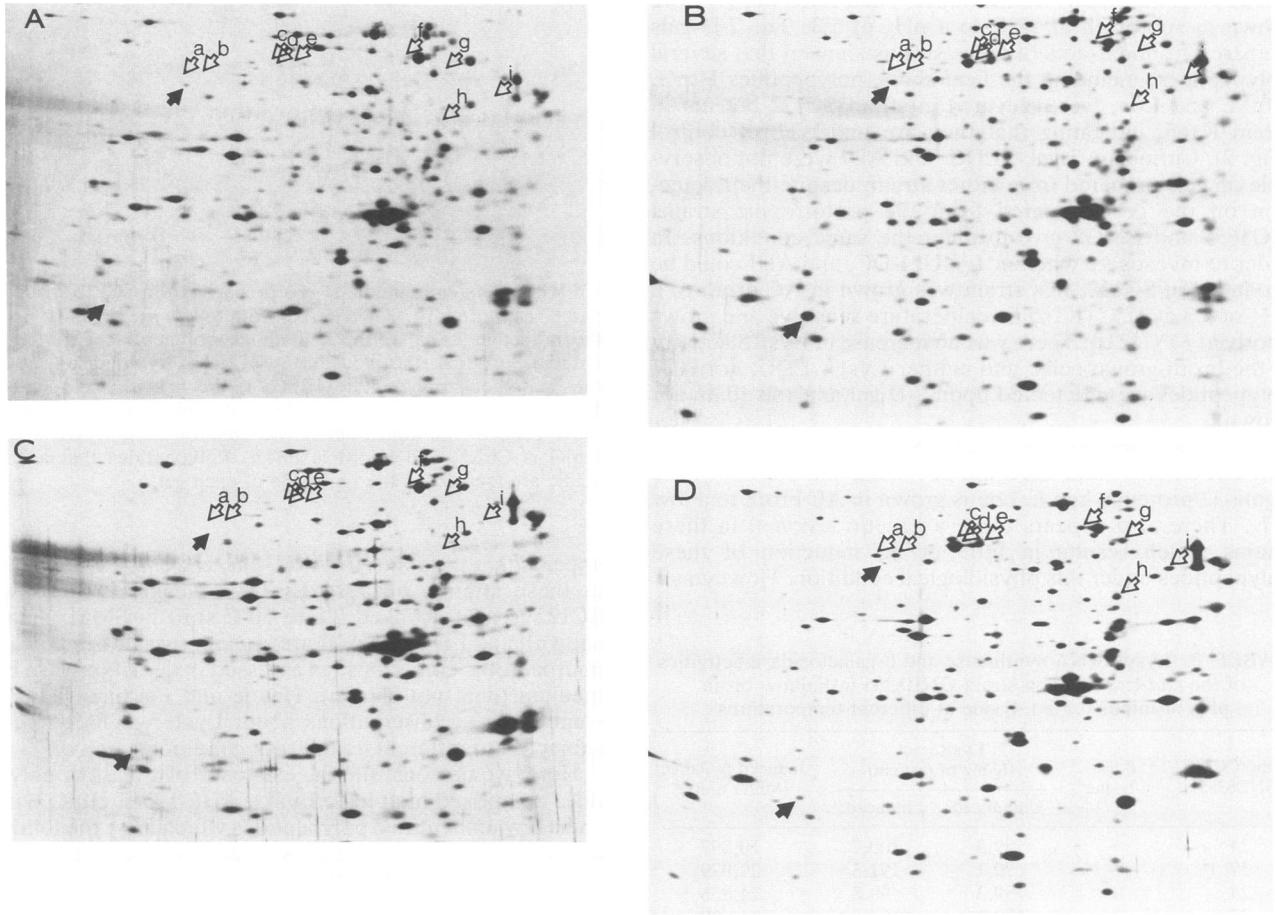

FIG. 1. Silver-stained 2-D gels prepared from MG1655. Cells were grown in SMM at 24°C (A), AC broth to low pH_o at 24°C (B), SMM at 37°C (C), and AC broth to low pH_o at 37°C (D). Open arrows point to the positions of polypeptides that appear to be induced better at 37°C (D) than at 24°C (B). a and b, the inducible LDC; f, the heat-shock polypeptide HtpM; g, the inducible ADI; h, the inducible lysyl-tRNA synthetase (LysU); i, the heat-shock polypeptide HtpG. Polypeptides c, d, and e were not identified. In the alphanumeric designation (25), LysU is D60.5, HtpG is C62.5, and HtpM is F84.1. Solid arrows point to the positions of two polypeptides that appear to be better induced at 24°C (B) than at 37°C (D). Polypeptides that are more acidic are located on the right side of each gel.

TABLE 2. Lysyl-tRNA synthetase and β-galactosidase activities of the *adi*::*lac* and *cadA*::*lac* fusion strains GNB7145 and GNB8385 at different temperatures

Strain and temp (°C)	LysRS act (U/mg of protein)		Induced β-Gal act (Miller units)[a]
	Uninduced[b]	Induced[c]	
GNB7145			
24	68.8	64.7	82.0
28	58.4	105.2	202.0
37	64.2	168.6	432.0
GNB8385			
24	74.3	93.5	21.0
28	79.0	119.4	148.0
37	71.5	160.0	169.0

[a] β-Galactosidase (β-Gal) activity in AC broth at pH_o 5.2 to 5.3. Uninduced β-Gal activity in SMM at pH_o 6.7 to 6.8 was less than 1 Miller unit.
[b] LysRS activity in SMM at pH_o 6.7 to 6.8.
[c] LysRS activity in AC broth at pH_o 5.2 to 5.3.

growth condition, LysRS activity is higher than that seen in AC broth (1.7-fold increase over the uninduced control) (Table 3); presumably, this is the result of the microaerobic growth conditions employed in these experiments (16).

The isogenic strains SC122 (*htpR*$^+$) and K165 (*htpR*) were grown in AC broth at 28°C to a pH_o of 5.3. The 2-D gels prepared from extracts of these strains showed that several polypeptides, including the heat-shock polypeptides HtpG, HtpM, and Lon, were elevated in strain SC122 but not in strain K165, indicating that they are under HtpR control (Fig. 2). Curiously, LysU, LDC, and ADI were not observable on gels prepared from either strain, despite their detection on the gels prepared from the prototrophic strains MG1655 and EMG2 grown under the same conditions. In order to investigate whether LysU, LDC, and ADI could be produced in SC122, this strain was grown in AC broth to a pH_o of 5.3 at 37°C (K165 is temperature sensitive and grows poorly at 37°C [21]). There was no increase in LysRS activity in the broth-grown cells, and neither LysU, LDC, nor ADI polypeptides were detected upon 2-D gel analysis (data not shown).

These experiments with strains SC122 and K165 indicate that LysU, ADI, and LDC are not under the control of the sigma-32 protein when *E. coli* is grown in AC broth to a low pH_o. There also appears to be a genetic lesion(s) in these strains which results in little or no induction of these polypeptides under this physiological condition. However, it

TABLE 3. Lysyl-tRNA synthetase and β-galactosidase activities of the *cadA*::*lac* fusion strain GNB8385 in Falkow broth plus or minus 0.5% L-lysine at different temperatures

Temp (°C) of GNB8385	0.5% L-lysine[a]	LysRS act (U/mg of protein)		Induced β-Gal act (Miller units)[b]
		Uninduced[c]	Induced[d]	
24	−	59.3	100.7	10,397
37	−	70.1	191.3	29,979
24	+	59.3	99.8	24,836
37	+	70.1	154.7	34,599

[a] −, without 0.5% L-lysine; +, with 0.5% L-lysine.
[b] β-Galactosidase (β-Gal) activity in Falkow broth plus or minus 0.5% L-lysine at pH_o 5.5. Uninduced β-Gal activity in SMM at pH_o 6.7 to 6.8 was less than 1 Miller unit.
[c] LysRS activity in SMM at pH_o 6.7 to 6.8.
[d] LysRS activity in Falkow broth plus or minus 0.5% L-lysine at pH_o 5.5.

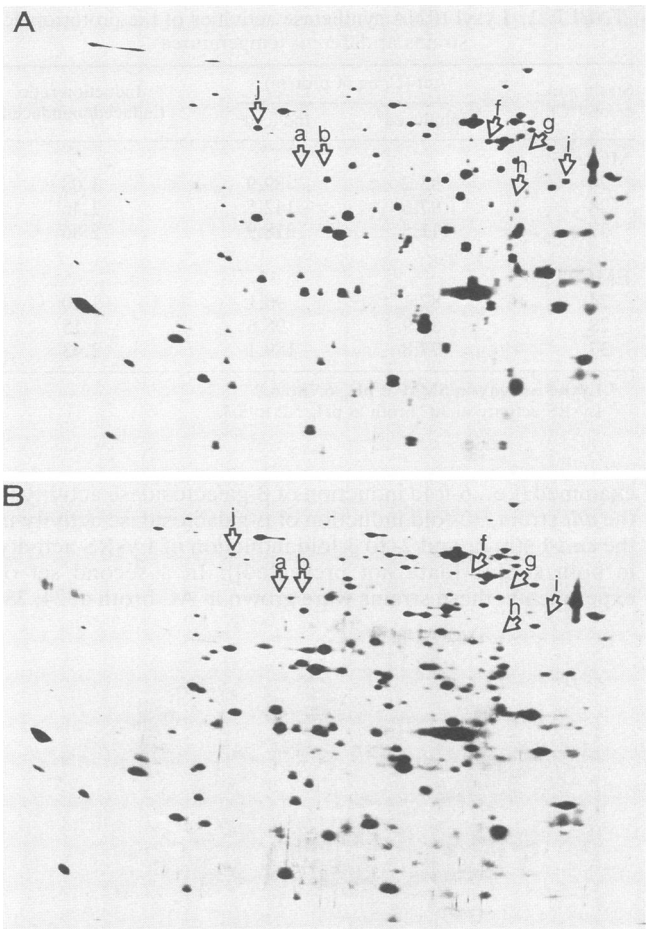

FIG. 2. Silver-stained 2-D gels prepared from SC122 (*htpR*$^+$) (A) and K165 (*htpR*) (B) grown in AC broth to low pH_o at 28°C. a and b, the inducible LDC; f, the heat-shock polypeptide HtpM; g, the inducible ADI; h, the inducible lysyl-tRNA synthetase (LysU); i, the heat-shock polypeptide HtpG; j, the heat-shock polypeptide Lon. The positions of polypeptides c, d, and e are not indicated on these gels. In the alphanumeric designation (25), LysU is D60.5, HtpG is C62.5, and HtpM is F84.1. Polypeptides that are more acidic are located on the right side of each gel.

appears that the genes for *lysU*, *adi*, and *cadA* are functional in these strains, because LysU is weakly induced when SC122 is heat shocked (28 to 50°C shift in SMM; data not shown), and LDC and ADI are apparently synthesized under microaerobic conditions, as assessed in decarboxylase broth medium (data not shown). Heyde and Portalier (11) made some of these observations about LysU when *E. coli* was subjected to different acid-stress conditions.

Here, we demonstrate the existence of a class of polypeptides comprised of at least LysU, ADI, LDC, HtpG, HtpM, and a few unidentified polypeptides which have the following characteristics. They are present at low levels or are undetectable in the SMM cultures but are induced in a temperature-dependent fashion in cultures grown in AC broth to low pH_o. It should be noted that the induction observed may not be due solely to low pH. The fact that HtpG and HtpM, but not LysU, ADI, and LDC, are under *htpR* control suggests that their genes are components of a stimulon (20). The studies with Falkow medium further demonstrate that there is temperature-dependent synthesis of LysU, ADI, and LDC

and that a high concentration of L-lysine can partially override the temperature-dependent control on *cadA* expression. Finally, another class of polypeptides that appear to be induced in AC broth at low pH_o but are found at greater amounts at the lower temperatures (i.e., at 24 or 28°C) was observed on the 2-D gels. Two members of this class are shown in Fig. 1.

It is well established that the combination of low environmental pH and temperature is synergistically bactericidal. Therefore, it is possible that at low external pH_o, *E. coli* proteins, such as LDC and ADI, which have been implicated in the alkalinization of an acidic cellular environment (5, 6, 28), as well as LysU, HtpG, and HtpM, which are documented cell stress proteins (21, 22), would be of greater necessity at 37°C than at lower temperatures. These results may be relevant to a major habitat of *E. coli*, the mammalian colon, in which the temperature is 37°C, the pH has been reported to be as low as 5.0 (18), and a complex assortment of nutrients is readily available.

We thank St. John's University for supporting this work.

We thank Barbara J. Bachmann for sending us the prototrophic strains used in this study, Richard A. Lockshin for his helpful discussion of the manuscript, and Mahassti Hassani for her technical assistance.

REFERENCES

1. **Auger, E. A., K. E. Redding, T. Plumb, L. C. Childs, S.-Y. Meng, and G. N. Bennett.** 1989. Construction of *lac* fusions to the inducible arginine and lysine decarboxylase of *Escherichia coli* K-12. Mol. Microbiol. **3**:1–12.
2. **Davis, B. D., and E. S. Mingioli.** 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. **60**:17–28.
3. **Foster, J., and H. Hall.** 1990. Adaptive acidification tolerance response of *Salmonella typhimurium*. J. Bacteriol. **172**:771–778.
4. **Foster, J. W., and H. K. Hall.** 1991. Inducible pH homeostasis and the acid tolerance response of *Salmonella typhimurium*. J. Bacteriol. **173**:5129–5135.
5. **Gale, E. F.** 1946. The bacterial amino acid decarboxylases. Adv. Enzymol. **6**:1–32.
6. **Gale, E. F., and H. M. R. Epps.** 1942. The effect of the pH of the medium during growth on the enzyme activities of bacteria (*Escherichia coli* and *Micrococcus lysodiekticus*) and the biological significance of the changes produced. Biochem. J. **36**: 600–619.
7. **Goodson, M., and R. J. Rowbury.** 1989. Habituation to normally lethal acidity by prior growth of *Escherichia coli* at a sublethal pH value. Lett. Appl. Microbiol. **8**:77–79.
8. **Goodson, M., and R. J. Rowbury.** 1989. Resistance of acid-habituated *Escherichia coli* to organic acids and its medical and applied significance. Lett. Appl. Microbiol. **8**:211–214.
9. **Grossman, A. D., J. W. Erickson, and C. A. Gross.** 1984. The *htpR* gene product of *E. coli* is a sigma factor for heat-shock promoters. Cell **38**:383–390.
10. **Hassani, M., M. V. Saluta, G. N. Bennett, and I. N. Hirshfield.** 1991. Partial characterization of a *lysU* mutant of *Escherichia coli* K-12. J. Bacteriol. **173**:1965–1970.
11. **Heyde, M., and R. Portalier.** 1990. Acid shock proteins of *Escherichia coli*. FEMS Microbiol. Lett. **69**:19–26.
12. **Hickey, E. W., and I. N. Hirshfield.** 1990. Low-pH-induced effects on patterns of protein synthesis and on internal pH in *Escherichia coli* and *Salmonella typhimurium*. Appl. Environ. Microbiol. **56**:1038–1045.
13. **Hirshfield, I. N., P. L. Bloch, R. A. VanBogelen, and F. C. Neidhardt.** 1981. Multiple forms of lysyl-transfer ribonucleic acid synthetase in *Escherichia coli* K-12. J. Bacteriol. **146**:345–351.
14. **Hirshfield, I. N., C. Liu, and F.-M. Yeh.** 1977. Two modes of metabolic regulation of lysyl-transfer ribonucleic acid synthetase in *Escherichia coli* K-12. J. Bacteriol. **131**:589–597.
15. **Hirshfield, I. N., R. Tenreiro, R. A. VanBogelen, and F. C. Neidhardt.** 1984. *Escherichia coli* K-12 lysyl-tRNA synthetase mutant with a novel reversion pattern. J. Bacteriol. **158**:615–620.
16. **Leveque, F., M. Gazeau, M. Fromant, S. Blanquet, and P. Plateau.** 1991. Control of *Escherichia coli* lysyl-tRNA synthetase expression by anaerobiosis. J. Bacteriol. **173**:7903–7910.
17. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
18. **Mallett, A. K., C. A. Beane, and I. R. Rowland.** 1989. The influence of incubation pH on the activity of rat and human gut flora enzymes. J. Appl. Microbiol. **66**:433–437.
19. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Neidhardt, F. C., J. L. Ingraham, and M. Schaechter.** 1990. Physiology of the bacterial cell. Sinauer Associates, Inc., Sunderland, Mass.
21. **Neidhardt, F. C., and R. A. VanBogelen.** 1981. Protein regulatory gene for temperature-controlled proteins in *Escherichia coli*. Biochem. Biophys. Res. Commun. **100**:894–900.
22. **Neidhardt, F. C., and R. A. VanBogelen.** 1987. Heat shock response, p. 1334–1345. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
23. **Novick, R. P., and W. K. Maas.** 1961. Control by endogenously synthesized arginine of the formation of ornithine transcarbamylase in *Escherichia coli*. J. Bacteriol. **81**:236–240.
24. **O'Farrell, P. H.** 1975. High resolution two-dimensional gels of proteins. J. Biol. Chem. **250**:4007–4021.
25. **Phillips, T. A., V. Vaughn, P. L. Bloch, and F. C. Neidhardt.** 1987. Gene-protein index of *Escherichia coli* K-12, 2nd ed., p. 919–966. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
26. **Raja, N., M. Goodson, W. C. M. Chui, D. G. Smith, and R. J. Rowbury.** 1991. Habituation to acid in *Escherichia coli*: conditions for habituation and its effects on plasmid transfer. J. Appl. Bacteriol. **70**:59–65.
27. **Slonczewski, J. L., T. N. Gonzalez, F. M. Bartholomew, and N. J. Holt.** 1987. *Mu* d-directed *lacZ* fusions regulated by low pH in *Escherichia coli*. J. Bacteriol. **169**:3001–3006.
28. **White, S., F. E. Tuttle, D. Blankenhorn, D. C. Dosch, and J. L. Slonczewski.** 1992. pH dependence and gene structure of *inaA* in *Escherichia coli*. J. Bacteriol. **174**:1537–1543.
29. **Wray, W., T. Boulikas, V. P. Wray, and R. Hancock.** 1981. Silver staining of proteins in polyacrylamide gels. Anal. Biochem. **118**:197–203.