Invited Review

Bacterial strategies to inhabit acidic environments

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Bacteria can inhabit a wide range of environmental conditions, including extremes in pH ranging from 1 to 11. The primary strategy employed by bacteria in acidic environments is to maintain a constant cytoplasmic pH value. However, many data demonstrate that bacteria can grow under conditions in which pH values are out of the range in which cytoplasmic pH is kept constant. Based on these observations, a novel notion was proposed that bacteria have strategies to survive even if the cytoplasm is acidified by low external pH. Under these conditions, bacteria are obliged to use acid-resistant systems, implying that multiple systems having the same physiological role are operating at different cytoplasmic pH values. If this is true, it is quite likely that bacteria have genes that are induced by environmental stimuli under different pH conditions. In fact, acid-inducible genes often respond to another factor(s) besides pH. Furthermore, distinct genes might be required for growth or survival at acid pH under different environmental conditions because functions of many systems are dependent on external conditions. Systems operating at acid pH have been described to date, but numerous genes remain to be identified that function to protect bacteria from an acid challenge. Identification and analysis of these genes is critical, not only to elucidate bacterial physiology, but also to increase the understanding of bacterial pathogenesis.

Key Words——acidic environment; acid-induction; adaptation; bacteria; cytoplasmic pH; gene regulation

Introduction

Bacteria are found in all areas of the earth's surface, including the poles. Their habitat encompasses a wide range of harsh environments, including extreme acidic and basic conditions (pH range of 1 to 11) (Padan et al., 1981). Since enzymatic activity is dependent on pH, changes in cytoplasmic pH disturb cellular metabolism, seriously challenging bacterial life. Bacteria have evolved strategies for surviving under these extreme pH conditions. Acid resistance is especially important for pathogenic bacteria growing under the strong acid stress found in the mammalian stomach

and in acidic phagosomes following infection.

1. Measurement of Bacterial Cytoplasmic pH

The primary mechanism for acid resistance is the maintenance of bacterial cytoplasmic pH. If cytoplasmic pH can be kept constant, cellular metabolism is not disturbed by pH changes in the surroundings. Various methods to measure bacterial cytoplasmic pH have been developed (Kashket, 1985). The first method is to use acidic and basic compounds as probes to measure pH. The data obtained by these probes showed that the cytoplasm of *Escherichia coli* is maintained at pH 7.7 within a wide range of medium pH (Padan et al., 1976). This method is based on the assumption that probes are not bound to cellular materials in the cytoplasm. Many negatively-charged intra-

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cellular molecules, however, can bind basic compounds. Castle et al. (1986) reported that nearly half of all sodium ions are bound in the cytoplasm, suggesting that basic probes for pH measurement may also be bound to internal molecules. In fact, the binding of the probe methylamine to RNA was demonstrated in vitro (Mugikura et al., 1990). It is well known that many basic probes used for pH measurement bind to outer cell surfaces. Because of this binding, it appears that a large fraction of basic probes is not free in the cytoplasm, resulting in decreased accuracy. This inaccuracy increases with increased pH because cytoplasmic molecules become more negatively charged at alkaline pH.

A method to measure cytoplasmic pH by nuclear magnetic resonance (NMR) has been developed. By the use of this technique, an internal *E. coli* pH of 7.6 ± 0.2 has been measured in media of pH values ranging from 5.5 to 9.0 (Slonczewski et al., 1981). But this method has several drawbacks. First, since the NMR signal is weak, extremely high concentrations of cells are required. Second, *E. coli* ferments substantial amounts of acids and bases at alkaline and acid pH, respectively. Therefore it is difficult to keep the medium pH constant at high cell densities. Third, increased fermented compound levels prevent further extrusion, resulting in internal accumulation of such compounds leading to cytoplasmic pH changes over the capacity of internal pH homeostasis.

Mugikura et al. (1990) measured the cytoplasmic pH of *E. coli* growing at various medium pH values by using acidic probes. The binding of acidic probes to internal molecules is negligible because the amount of positively charged molecules is low at near neutral pH. Their data showed that cytoplasmic pH was kept constant within the narrow range of medium pH from 6.0 to 7.8, and that the cytoplasmic pH decreased and increased at medium pH below 6.0 and above 7.8, respectively (Fig. 1). The cytoplasmic pH was also measured by using a fluorescence dye. These results indicated that the pH was maintained at 7.8 to 7.9 only within a medium pH range of 6.0 to 7.5 in *E. coli* (Shechter et al., 1982). The internal pH increased to 8.3 at a medium pH of 8.3, and the cytoplasm was acidified to 7.6 at medium pH 5.5 (Shechter et al., 1982). The cytoplasmic pH values of growing cells were somewhat lower than those observed with cells resuspended in a buffer at any external pH tested (unpublished observations). This may be the reason to

Fig. 1. Cytoplasmic pH of *E. coli* growing in a medium of various pH values.

For detailed experimental procedures, see Mugikura et al. (1990), from which these data were drawn. A broken line represents the intracellular pH equal to the medium pH. *E. coli* can grow within the pH range represented by the gray bar.

account for the difference between cytoplasmic pH values shown in Fig. 1 and data reported by other groups. In all cases, it has been demonstrated that *E. coli* can maintain cytoplasmic pH at a constant value when growing in a medium of near neutral pH.

2. Regulatory Mechanisms for Cytoplasmic pH Homeostasis

The regulatory mechanism for bacterial cytoplasmic pH has been studied for a quarter century, but many issues remain unresolved. It is now generally accepted that cytoplasmic pH is regulated by proton extrusion and cytoplasmic accumulation of potassium ions under acidic growth conditions (Booth, 1985; Padan et al., 1981). To maintain constant pH, the regulatory system may be expected to be less active when the cytoplasm pH value is favorable for cellular metabolism. If the cytoplasm is acidified from the favorable pH range, a rapid increase in proton-extruding activity would be required to return the cytoplasmic pH to the original value. Therefore any regulatory system for maintaining cytoplasmic pH should be responsive to pH.

The mechanism for cytoplasmic pH regulation has been well defined in enterococci. This bacterium grows optimally when the cytoplasmic pH is about 7.6 (Kobayashi, 1987). This species does not have the respiratory chain, and protons are extruded via F-type proton translocating ATPases (Harold and Altendorf, 1974). Various research approaches have provided insights into the regulatory mechanism of maintaining

cytoplasmic pH in this species. In acid medium, the cytoplasm is maintained at pH 7.6 by proton extrusion via the H^+ -ATPase and potassium ion accumulation (Kobayashi, 1987). Mutant strains deficient in the H^+ -ATPase are unable to grow at pH below 6.5 (Kobayashi, 1985), and wild type cells grow within the wide range of medium pH from 4.0 to 10.5. These results indicate that a regulation of cytoplasmic pH is essential for growth under acidic environments and that the H^+ -ATPase is required.

Membrane levels of enterococcal ATPase increase in response to decreased cytoplasmic pH. Elevated amounts of the ATPase reverse acidification of the cytoplasm by proton extrusion (Abrams and Jensen, 1984; Kobayashi et al., 1984). After the acidified cytoplasm is alkalinized to 7.6, the enzyme level decreases (Kobayashi et al., 1986; Suzuki and Kobayashi, 1989). At pH levels above 7.6, ATPase activity declines dramatically, preventing alkalinization in acidic medium (Kobayashi et al., 1986; Suzuki and Kobayashi, 1989). Interestingly, membrane levels of ATPase are regulated at the level of assembly (Arikado et al., 1999); less than 50% of subunits synthesized assemble into functional enzyme. The fraction of assembled subunits increases at low pH, resulting in increased amounts of membrane-bound enzyme. Increased H^+ -ATPase levels are also observed in *Salmonella enterica* serovar Typhimurium (Foster and Hall, 1990, 1991), *Streptococcus mutans* (Smith et al., 1996), *Lactobacillus acidophilus* (Kullen and Klaenhammer, 1999), and *Lactococcus lactis* (O'Sullivan and Condon, 1999). The transcription of the H^+ -ATPase was stimulated in *L. acidophilus* (Kullen and Klaenhammer, 1999), but not in *S. mutans* (Smith et al., 1996), indicating that various mechanisms are used to elevate H^+ -ATPase levels to allow growth at acid pH.

Respiring bacteria (e.g., *E. coli*) extrude protons via the respiratory chain. A proton motive force generated by this chain is used for ATP synthesis, membrane transport, and cell motility (Harold, 1982). Many data support that both proton extrusion via the respiratory chain and potassium ion accumulation mediated by specific transport systems participate in the regulation of cytoplasmic pH in acidic medium (reviewed in Booth, 1985). If cytoplasmic pH is regulated by these two systems, at least one of the systems should be affected by pH, as discussed above. However, the respiratory chain is active within the wide range of medium

pH from 5.0 to 8.5 (Padan et al., 1976) and there has been no report to show that an accumulation of potassium ions declines at high pH.

If, on the other hand, another system mediates an uptake of protons at cytoplasmic pH above 7.6, further alkalinization of the cytoplasm could be prevented. It has been proposed that when the cytoplasm is alkalinized beyond 7.6, either the Na^+/H^+ or K^+/H^+ antiporter takes up protons to compensate for the cytoplasmic alkalinization (Padan and Schuldiner, 1986, 1993, 1994; Padan et al., 1981). *E. coli* has three systems (NhaA, NhaB, and ChaA) to extrude sodium ions (Sakuma et al., 1998). Mutant bacteria deficient in all three antiporters grow, as well as wild type, at any pH tested in medium containing low levels of sodium ions. No active extrusion of sodium ions was observed under these conditions (Sakuma et al., 1998), ruling out the possibility that an Na^+/H^+ antiporter plays a role in pH regulation.

An alternative explanation may be that a K^+/H^+ antiporter regulates cytoplasmic pH when the Na^+/H^+ antiporter is inoperative. However, regulation in this manner appears to simply waste energy; the respiratory chain extrudes protons continuously and the extruded protons are taken up again in an energy-requiring process. *E. coli* has two extrusion systems for potassium ions that are active only in cells treated with a reducing agent (Elmore et al., 1990; Ness and Booth, 1999). Potassium ions are removed from the cytoplasm when cells growing at low pH are transferred to alkaline medium of pH above 8 (Yamasaki et al., 1980). A similar efflux, however, was not observed after the addition of an energy source under iso-osmolar conditions (unpublished results).

Futatsugi et al. (1997) found that an *E. coli* mutant deficient in the respiratory chain grew at a somewhat slower rate than the wild type at any pH tested. It is interesting that the internal pH of the mutant was maintained at a value of about 7.1 in a medium of pH 6, suggesting that this bacterium has an unidentified system for cytoplasmic pH regulation functioning without respiration. Thus the mechanism for the cytoplasmic regulation is still unclear in *E. coli*.

3. How Do Bacteria Adapt to Changes in Cytoplasmic pH?

Many genes have been reported to be expressed in a regulated fashion at acid pH in bacteria (Olson,

1993). If the cytoplasmic pH is kept constant, gene expression should be triggered by lowering medium pH and not by changes in cytoplasmic pH. It is unlikely that all regulators of these genes are directly affected by medium pH. The range of medium pH allowing *E. coli* to grow is 4.5 to 9.0. This range is wider than the medium pH range in which cytoplasmic pH is kept constant, as shown previously (Mugikura et al., 1990; Shechter et al., 1982; see Fig. 1). Based on these observations, a novel notion was proposed that bacteria have strategies to survive even if the cytoplasm is acidified or alkalinized from the optimum pH for growth (Kobayashi et al., 1999). Studies of these adaptive systems are very important, not only for elucidating bacterial physiology, but also to promote our understanding of bacterial pathogenesis.

It is well known that bacteria have multiple systems for carrying out essential functions. For example, *E. coli* has three extrusion systems for sodium ions—NhaA, NhaB, and ChaA—as described above. Sakuma et al. (1998) demonstrated that NhaB works in neutral and acid media, and ChaA extrudes sodium ions in alkaline medium. When the activities of both systems are not enough to maintain low internal levels of sodium ions in high concentrations of external sodium ions, NhaA is induced. The induction of NhaA appears to be more significant at high pH because the maximum activity of ChaA at high pH is less than that of NhaB at low pH. This may relate to the optimum activity of NhaA at pH above 8 (Gerchman et al., 1993; Taglicht et al., 1991).

E. coli has four systems for increasing intracellular potassium ion concentrations, Kdp, TrkA, Kup, and TrkF (Epstein et al., 1993). The activity of the major system, TrkA, declines rapidly at acid pH. In contrast, Kup has an optimum activity at acid pH and is the main system for potassium ion accumulation under hyperosmotic stress at pH 5.5 (Trchounian and Kobayashi, 1999). Therefore Kup seems to work to compensate for the decrease in the activity of TrkA at acid pH. The affinity of Kdp for potassium ions is higher than the other systems, and this system is thought to function in medium containing a very low amount of potassium ions (Epstein et al., 1993).

It is now well accepted that levels of the outer membrane porins, OmpC and OmpF, are regulated by medium osmolarity in bacteria (Csonka, 1989; Mizuno and Mizushima, 1990; Nikaido and Vaara, 1985). The levels are also affected by medium pH (Heyde and

Portalier, 1987; Heyde et al., 2000; Sato et al., 2000; Thomas and Booth, 1992). OmpC is expressed under hyperosmosis, and the level of OmpF decreases with the increase in external osmolarity in neutral and alkaline media. In contrast at high pH, both OmpC and OmpF are expressed in medium containing minimum amounts of nutrients for growth, and the expression of both porins decreases under hyperosmotic stress at acid pH below 6 (Sato et al., 2000). Porin expression correlates with the level of phosphorylated OmpR (OmpR-Pi), which is controlled by phosphorylation and dephosphorylation via EnvZ, a sensor of external osmolarity (Csonka, 1989; Mizuno and Mizushima, 1990; Nikaido and Vaara, 1985). Recent data obtained by our group suggests that the binding affinity of OmpR-Pi to the regulatory region of *ompC* increases at low pH, resulting in elevated OmpC expression at a low level of OmpR-Pi under hypo-osmosis (unpublished results). Porins were found to be essential for the growth of *E. coli* in the presence of limited amounts of nutrients at acid pH, but not at high pH (Sato et al., 2000). Therefore this differential regulation of porin expression at different pH values is quite significant for bacterial physiology.

Many acid-inducible genes respond to other environmental stimuli besides pH. *E. coli* has multiple amino acid decarboxylases, namely, biosynthetic and biodegradative enzymes (Tabor and Tabor, 1985). Biodegradative ornithine, lysine, and arginine decarboxylases are expressed at acid pH (Meng and Bennett, 1992a,b; Stim and Bennett, 1993; Tabor and Tabor, 1985; Watson et al., 1992). Because amines produced by these decarboxylases neutralize acids, it has been proposed that these decarboxylases alkalinize acid medium to increase the growth rate (Tabor and Tabor, 1985). However, the expression of these decarboxylases is also stimulated under oxygen-limiting conditions (Meng and Bennett, 1992a,b; Stim and Bennett, 1993; Watson et al., 1992). Takayama et al. (1994) demonstrated that carbon dioxide (or its derivatives) suppresses the expression of the *cad* operon (encoding lysine decarboxylase and lysine/cadaverine exchanger). The supply of carbon dioxide from air is limited at acid pH. The major metabolic pathway that produces carbon dioxide is the TCA cycle, and TCA activity declines when oxygen is limited through linkage with the respiratory chain. Reams et al. (1997) showed that blocking the respiratory chain derepressed a *cadA-lacZ* fusion gene under aerobic condi-

tions. The induction of lysine decarboxylase increased the growth yield in a mutant deficient in heme synthesis, but not in the wild type in well-buffered acid medium (Futatsugi et al., 1997). In their experiments, no significant difference was found in medium alkalization during growth between the two strains. These results support the previous proposal by Boeker and Snell (1972) that amino acid decarboxylases serve to control the level of carbon dioxide required for metabolic pathways, such as the urea cycle or the synthetic pathway of pyrimidine nucleosides. It would be advantageous for bacterial life to have multiple amino acid decarboxylases with optimal activities at various pH values. A metabolic supply of carbon dioxide is required, not only in acid medium, but also at alkaline pH, when its levels decrease.

The gene designated *asr* was induced at low pH under phosphate-limiting conditions in *E. coli* (Suziedlien et al., 1999). This gene was also controlled by the PhoB/PhoR two-component system, which is known to regulate the *pho* regulon under phosphate-limiting conditions in neutral medium (Suziedlien et al., 1999). These data lead us to assume that the PhoB/PhoR system induces *asr* expression under phosphate-limiting conditions at acid pH instead of one of the *pho* regulons working at high pH. Acid-inducible genes related to phosphate utilization were also reported in *S. enterica* serovar Typhimurium (Bearson et al., 1998). Three systems for magnesium ion accumulation, *CorA*, *MgtA*, and *MgtB*, were found in *S. enterica* serovar Typhimurium (Hmiel et al., 1989; Smith et al., 1998). The activity of constitutive *CorA* was high at acid pH, and the induction of *mgtA* was abolished at pH 5.2 and the expression of *mgtCB* encoding *MgtB* was low in acid medium (Smith et al., 1998), implying that *CorA* works at acid pH, but that *MgtA* and *MgtB* operate mainly at high pH. *E. coli* has two hydrogenases encoded by *hya* and *hyb*. The *hya* gene was induced at medium pH below 6, and *E. coli* expressed the *hyb* gene within the wide range of medium pH. But *hyb* expression levels were maximum at alkaline pH (King and Przybyla, 1999).

As described above, bacteria seem to have multiple systems sensing the same environmental factor(s) at different pH values. If an expression of a given gene is dependent on cytoplasmic pH, its level may be unchanged at medium pH values from 6.0 to 7.8, since the cytoplasmic pH is kept constant within this range (Fig. 1). The cytoplasmic pH-dependent expression of

any gene can be observed only at medium pH values below 6.0 or above 7.8.

4. Survival under Acid Stress

Neutrophilic bacteria struggle to maintain viability in the extreme acid medium of pH below 3. Increased stability is observed, however, if cells are pretreated at pH around 5. Recent investigations into the mechanism for this acid tolerance response (ATR) have been performed mainly in the group of J. W. Foster with *E. coli* and *S. enterica* serovar Typhimurium. The induction of arginine decarboxylase (*Adi*) or glutamate decarboxylase (*Gad*) promoted acid resistance (Castanie-Cornet et al., 1999). *E. coli* has two glutamate decarboxylases, *GadA* and *GadB*. One of the two *Gads* prevented the death of *E. coli* at pH 2.5, but survival at pH 2 required both *Gads* (Castanie-Cornet et al., 1999).

The way in which amino acid decarboxylases protect against an acid challenge is unknown. An accumulation of γ -aminobutyric acid (GABA) plays an important role in osmotic adaptation (Measures, 1975; Ogahara et al., 1995), and the regulation of internal osmolarity with GABA might be essential for protection at acid pH. As discussed above, the main physiological role of amino acid decarboxylases is to supply carbon dioxide metabolically. The maintenance of carbon dioxide at appropriate levels is very likely to be necessary for survival under acid stress.

Helicobacter pylori, a pathogenic bacteria involved in gastric ulcers, produces a urease that converts urea to ammonium and carbon dioxide. This urease has been shown to be central to the pathogenesis of this bacterium (Clyne et al., 1995; Krishnamurthy et al., 1998; Sjostrom and Larsson, 1996). One possibility is that ammonium produced through urease neutralizes the acidic medium and protects bacteria against cell death. However, this neutralization occurs only at high cell densities, and a cell number of bacteria colonizing the stomach is not sufficient to neutralize the secreted acid. The amount of urea present in the mammalian stomach is limited, provided mainly through an arginase conversion of arginine to ornithine and urea. McGee et al. (1999) showed that a mutant deficient in arginase was sensitive to acid in vitro, but this enzyme was not essential for the colonization of mice. Furthermore, amino acid deaminases play little or no role in protecting against acid exposure (McGee et al., 1999).

Such amino acid deaminases produce ammonium, but not carbon dioxide. These data again support a model in which the production of carbon dioxide is essential for growth and survival at acid pH.

A mutant of *S. enterica* serovar Typhimurium deficient in the sigma factor sigma S (σ^S) is sensitive to acid, suggesting that a σ^S -dependent gene(s) may be involved in ATR (Lee et al., 1995). The requirement of σ^S for acid resistance was also reported in *E. coli* and *Shigella flexneri* (Lin et al., 1996; Price et al., 2000; Small et al., 1994). Membrane cyclopropane fatty acid was found to have an essential role in acid resistance in *E. coli* (Chang and Cronan, 1999). The *cfa* gene, whose product synthesizes cyclopropane fatty acid, has two promoters (P1 and P2) dependent on σ^{70} and σ^{38} (also called σ^S), respectively (Eichel et al., 1999). The *cfa* expression was not affected significantly by the deletion of σ^S in the presence of both promoters. The expression of *gadA* and *gadB* genes was independent from σ^S (Castanie-Cornet et al., 1999). Thus the σ^S -dependent genes required for ATR induction remain to be clarified.

Medium factors also participate in the induction of acid tolerance. *E. coli* produced protease-sensitive compounds at alkaline pH that protected cells from acid stress (Rowbury, 1999; Rowbury et al., 1998). Contrary results, however, have also been reported. For example, the growth of *E. coli* at pH 8.0 produced an inhibitor of acid resistance (Castanie-Cornet et al., 1999). Taken together, these results suggest that ATR is dependent on environmental conditions. Therefore different genes appear to be required for growth and survival at low pH under different conditions.

5. Mechanism for pH-Dependent Gene Expression

An interesting question is how gene expression is regulated by pH. Although there is no direct evidence to date, the following explanations are plausible. 1) A global pH sensor binds to a common DNA sequence or responds to a pH-dependent change of DNA structure. 2) The binding constant of a regulatory protein (e.g., transcription factor) for each gene varies according to pH. 3) A factor regulates translation in a pH-dependent manner. 4) The assembly of functional units is regulated by pH. Until now, no pH-dependent changes in DNA structure under physiological conditions have been reported. A global sensor, if present, might bind to a common DNA sequence. Some acid-inducible

genes are controlled by σ^S , which is known to regulate gene expression at the stationary growth phase, indicating that σ^S is a candidate for the global sensor (Lee et al., 1995). It should be noted that the cytoplasm is acidified at the stationary phase, making it likely that σ^S -dependent genes might be controlled in a common fashion at both acid pH and the stationary phase. It is now evident that σ^S -independent genes are also induced at acid pH. The binding activity of phosphorylated OmpR is dependent on pH, as described above. In *E. hirae*, the membrane level of H⁺-ATPase is regulated by pH at the step of assembly (Arikado et al., 1999). Thus expression of each gene functioning under acid environments may be regulated in a different manner.

Protonation or deprotonation of some amino acid residue(s) of proteins could govern pH-dependent gene expression. Since the discovery of enzymes in the past century, it has been well documented that enzyme activity is dependent on pH. The mechanism of pH dependency, however, is still unknown for many enzymes. For example, the activity of NhaA, one of the *E. coli* systems for sodium ion extrusion, is optimal at alkaline pH (Gerchman et al., 1993; Taglicht et al., 1991). When the histidine at position 226 was replaced with arginine, its optimum activity shifted to low pH (Gerchman et al., 1993). The substitution of leucine at position 75 with arginine shifted the optimum activity to a low pH in similar fashion (Noumi et al., 1997), suggesting that many kinds of amino acid residues are involved in pH sensing. Further investigations into a pH sensor residue(s) of regulatory proteins should help the dissection of pH-dependent gene expression.

6. Concluding Remarks

Bacteria have two general strategies for adaptation to changes in environmental pH. One is to maintain cytoplasmic pH homeostasis and the other is to use systems resistant to acid and alkaline pH when the cytoplasm is acidified or alkalinized, respectively. Until now, numerous enzymes have been demonstrated to play important roles in growth and survival under an acid challenge, but most genes functioning at acid pH seem to be unidentified. The entire genome sequence of *E. coli* was recently reported (Blattner et al., 1997) and showed 4,288 open reading frames, only half of which have been previously described. Because almost all known genes have been identified from cells

growing at near neutral pH, many unidentified genes may function under acid or alkaline conditions and not at near neutral medium (Kobayashi et al., 1999). Further studies with *E. coli* growing in a medium of pH below 6 or above 8 should promote greater understanding of the physiological roles of the rest of the genes.

References

- Abrams, A. and Jensen, C. (1984) Altered expression of the H⁺ ATPase in *Streptococcus faecalis* membranes. *Biochem. Biophys. Res. Commun.*, **122**, 151–157.
- Arikado, E., Ishihara, H., Ehara, T., Shibata, C., Saito, H., Kakegawa, T., Igarashi, K., and Kobayashi, H. (1999) Enzyme level of enterococcal F₁F₀-ATPase is regulated by pH at the step of assembly. *Eur. J. Biochem.*, **259**, 262–268.
- Bearson, B. L., Wilson, L., and Foster, J. W. (1998) A low pH-inducible, PhoPQ-dependent acid tolerance response protects *Salmonella typhimurium* against inorganic acid stress. *J. Bacteriol.*, **180**, 2409–2417.
- Blattner, F. R., Plunkett, G., 3rd, Bloch, C. A., Perna, N. T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J. D., Rode, C. K., Mayhew, G. F., Gregor, J., Davis, N. W., Kirkpatrick, H. A., Goeden, M. A., Rose, D. J., Mau, B., and Shao, Y. (1997) The complete genome sequence of *Escherichia coli* K-12. *Science*, **277**, 1453–1474.
- Boeker, E. A. and Snell, E. E. (1972) Amino acid decarboxylase. In *The Enzymes*, 3rd ed., ed. by Boyer, P. D., Academic Press, New York, pp. 217–253.
- Booth, I. R. (1985) Regulation of cytoplasmic pH in bacteria. *Microbiol. Rev.*, **49**, 359–378.
- Castanie-Cornet, M. P., Penfound, T. A., Smith, D., Elliott, J. F., and Foster, J. W. (1999) Control of acid resistance in *Escherichia coli*. *J. Bacteriol.*, **181**, 3525–3535.
- Castle, A. M., Macnab, R. M., and Shulman, R. G. (1986) Measurement of intracellular sodium concentration and sodium transport in *Escherichia coli* by ²³Na nuclear magnetic resonance. *J. Biol. Chem.*, **261**, 3288–3294.
- Chang, Y. Y. and Cronan, J. E., Jr. (1999) Membrane cyclopropane fatty acid content is a major factor in acid resistance of *Escherichia coli*. *Mol. Microbiol.*, **33**, 249–259.
- Clyne, M., Labigne, A., and Drumm, B. (1995) *Helicobacter pylori* requires an acidic environment to survive in the presence of urea. *Infect. Immun.*, **63**, 1669–1673.
- Csonka, L. N. (1989) Physiological and genetic responses of bacteria to osmotic stress. *Microbiol. Rev.*, **53**, 121–147.
- Eichel, J., Chang, Y. Y., Riesenberger, D., and Cronan, J. E., Jr. (1999) Effect of ppGpp on *Escherichia coli* cyclopropane fatty acid synthesis is mediated through the RpoS sigma factor (σ^S). *J. Bacteriol.*, **181**, 572–576.
- Elmore, M. J., Lamb, A. J., Ritchie, G. Y., Douglas, R. M., Munro, A., Gajewska, A., and Booth, I. R. (1990) Activation of potassium efflux from *Escherichia coli* by glutathione metabolites. *Mol. Microbiol.*, **4**, 405–412.
- Epstein, W., Buurman, E., McLaggan, D., and Naprstek, J. (1993) Multiple mechanisms, roles and controls of K⁺ transport in *Escherichia coli*. *Biochem. Soc. Trans.*, **21**, 1006–1010.
- Foster, J. W. and Hall, H. K. (1990) Adaptive acidification tolerance response of *Salmonella typhimurium*. *J. Bacteriol.*, **172**, 771–778.
- Foster, J. W. and Hall, H. K. (1991) Inducible pH homeostasis and the acid tolerance response of *Salmonella typhimurium*. *J. Bacteriol.*, **173**, 5129–5135.
- Futatsugi, L., Saito, H., Kakegawa, T., and Kobayashi, H. (1997) Growth of an *Escherichia coli* mutant deficient in respiration. *FEMS Microbiol. Lett.*, **156**, 141–145.
- Gerchman, Y., Olami, Y., Rimon, A., Taglicht, D., Schuldiner, S., and Padan, E. (1993) Histidine-226 is part of the pH sensor of NhaA, a Na⁺/H⁺ antiporter in *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 1212–1216.
- Harold, F. M. (1982) Pumps and currents: A biological perspective. *Curr. Top. Membr. Transp.*, **16**, 485–516.
- Harold, F. M. and Altendorf, K. (1974) Cation transport in bacteria: K⁺, Na⁺ and H⁺. *Curr. Top. Membr. Transp.*, **5**, 1–50.
- Heyde, M., Laloi, P., and Portalier, R. (2000) Involvement of carbon source and acetyl phosphate in the external-pH-dependent expression of porin genes in *Escherichia coli*. *J. Bacteriol.*, **182**, 198–202.
- Heyde, M. and Portalier, R. (1987) Regulation of major outer membrane porin proteins of *Escherichia coli* K 12 by pH. *Mol. Gen. Genet.*, **208**, 511–517.
- Hmiel, S. P., Snively, M. D., Florer, J. B., Maguire, M. E., and Miller, C. G. (1989) Magnesium transport in *Salmonella typhimurium*: Genetic characterization and cloning of three magnesium transport loci. *J. Bacteriol.*, **171**, 4742–4751.
- Kashket, E. R. (1985) The proton motive force in bacteria: A critical assessment of methods. *Annu. Rev. Microbiol.*, **39**, 219–242.
- King, P. W. and Przybyla, A. E. (1999) Response of *hya* expression to external pH in *Escherichia coli*. *J. Bacteriol.*, **181**, 5250–5256.
- Kobayashi, H. (1985) A proton-translocating ATPase regulates pH of the bacterial cytoplasm. *J. Biol. Chem.*, **260**, 72–76.
- Kobayashi, H. (1987) Regulation of cytoplasmic pH in streptococci. In *Sugar Transport and Metabolism in Gram-positive Bacteria*, ed. by Reizer, J. and Peterkofsky, A., Ellis Horwood Ltd., Chichester, pp. 255–269.
- Kobayashi, H., Saito, H., Futatsugi, L., and Kakegawa, T. (1999) Cation movements at alkaline pH in bacteria growing without respiration. *Novartis Found. Symp.*, **221**, 235–242; discussion 242–245.
- Kobayashi, H., Suzuki, T., Kinoshita, N., and Unemoto, T. (1984) Amplification of the *Streptococcus faecalis* proton-translocating ATPase by a decrease in cytoplasmic pH. *J. Bacteriol.*, **158**, 1157–1160.
- Kobayashi, H., Suzuki, T., and Unemoto, T. (1986) Streptococcal cytoplasmic pH is regulated by changes in amount and

- activity of a proton-translocating ATPase. *J. Biol. Chem.*, **261**, 627–630.
- Krishnamurthy, P., Parlow, M., Zitzer, J. B., Vakil, N. B., Mobley, H. L. T., Levy, M., Phadnis, S. H., and Dunn, B. E. (1998) *Helicobacter pylori* containing only cytoplasmic urease is susceptible to acid. *Infect. Immun.*, **66**, 5060–5066.
- Kullen, M. J. and Klaenhammer, T. R. (1999) Identification of the pH-inducible, proton-translocating F_1F_0 -ATPase (*atp-BEFHAGDC*) operon of *Lactobacillus acidophilus* by differential display: Gene structure, cloning and characterization. *Mol. Microbiol.*, **33**, 1152–1161.
- Lee, I. S., Lin, J., Hall, H. K., Bearson, B., and Foster, J. W. (1995) The stationary-phase sigma factor σ^S (RpoS) is required for a sustained acid tolerance response in virulent *Salmonella typhimurium*. *Mol. Microbiol.*, **17**, 155–167.
- Lin, J., Smith, M. P., Chapin, K. C., Baik, H. S., Bennett, G. N., and Foster, J. W. (1996) Mechanisms of acid resistance in enterohemorrhagic *Escherichia coli*. *Appl. Environ. Microbiol.*, **62**, 3094–3100.
- McGee, D. J., Radcliff, F. J., Mendz, G. L., Ferrero, R. L., and Mobley, H. L. (1999) *Helicobacter pylori* *rocF* is required for arginase activity and acid protection in vitro but is not essential for colonization of mice or for urease activity. *J. Bacteriol.*, **181**, 7314–7322.
- Measures, J. C. (1975) Role of amino acids in osmoregulation of non-halophilic bacteria. *Nature*, **257**, 398–400.
- Meng, S. Y. and Bennett, G. N. (1992a) Regulation of the *Escherichia coli* *cad* operon: Location of a site required for acid induction. *J. Bacteriol.*, **174**, 2670–2678.
- Meng, S. Y. and Bennett, G. N. (1992b) Nucleotide sequence of the *Escherichia coli* *cad* operon: A system for neutralization of low extracellular pH. *J. Bacteriol.*, **174**, 2659–2669.
- Mizuno, T. and Mizushima, S. (1990) Signal transduction and gene regulation through the phosphorylation of two regulatory components: The molecular basis for the osmotic regulation of the porin genes. *Mol. Microbiol.*, **4**, 1077–1082.
- Mugikura, S., Nishikawa, M., Igarashi, K., and Kobayashi, H. (1990) Maintenance of a neutral cytoplasmic pH is not obligatory for growth of *Escherichia coli* and *Streptococcus faecalis* at an alkaline pH. *J. Biochem. (Tokyo)*, **108**, 86–91.
- Ness, L. S. and Booth, I. R. (1999) Different foci for the regulation of the activity of the KefB and KefC glutathione-gated K^+ efflux systems. *J. Biol. Chem.*, **274**, 9524–9530.
- Nikaido, H. and Vaara, M. (1985) Molecular basis of bacterial outer membrane permeability. *Microbiol. Rev.*, **49**, 1–32.
- Noumi, T., Inoue, H., Sakurai, T., Tsuchiya, T., and Kanazawa, H. (1997) Identification and characterization of functional residues in a Na^+/H^+ antiporter (NhaA) from *Escherichia coli* by random mutagenesis. *J. Biochem. (Tokyo)*, **121**, 661–670.
- Ogahara, T., Ohno, M., Takayama, M., Igarashi, K., and Kobayashi, H. (1995) Accumulation of glutamate by osmotically stressed *Escherichia coli* is dependent on pH. *J. Bacteriol.*, **177**, 5987–5990.
- Olson, E. R. (1993) Influence of pH on bacterial gene expression. *Mol. Microbiol.*, **8**, 5–14.
- O'Sullivan, E. and Condon, S. (1999) Relationship between acid tolerance, cytoplasmic pH, and ATP and H^+ -ATPase levels in chemostat cultures of *Lactococcus lactis*. *Appl. Environ. Microbiol.*, **65**, 2287–2293.
- Padan, E. and Schuldiner, S. (1986) Intracellular pH regulation in bacterial cells. *Methods Enzymol.*, **125**, 337–352.
- Padan, E. and Schuldiner, S. (1993) Na^+ transport systems in prokaryotes. In *Alkali Cation Transport Systems in Prokaryotes*, ed. by Bakker, E. P., CRC Press, Boca Raton, pp. 3–24.
- Padan, E. and Schuldiner, S. (1994) Molecular biology of Na^+/H^+ antiporters: Molecular devices that couple the Na^+ and H^+ circulation in cells. *Biochim. Biophys. Acta*, **1187**, 206–210.
- Padan, E., Zilberstein, D., and Rottenberg, H. (1976) The proton electrochemical gradient in *Escherichia coli* cells. *Eur. J. Biochem.*, **63**, 533–541.
- Padan, E., Zilberstein, D., and Schuldiner, S. (1981) pH homeostasis in bacteria. *Biochim. Biophys. Acta*, **650**, 151–166.
- Price, S. B., Cheng, C. M., Kaspar, C. W., Wright, J. C., DeGraves, F. J., Penfound, T. A., Castanie-Cornet, M. P., and Foster, J. W. (2000) Role of *rpoS* in acid resistance and fecal shedding of *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.*, **66**, 632–637.
- Reams, S. G., Lee, N., Mat-Jan, F., and Clark, D. P. (1997) Effect of chelating agents and respiratory inhibitors on regulation of the *cadA* gene in *Escherichia coli*. *Arch. Microbiol.*, **167**, 209–216.
- Rowbury, R. J. (1999) Acid tolerance induced by metabolites and secreted proteins, and how tolerance can be counteracted. *Novartis Found. Symp.*, **221**, 93–106; discussion 106–111.
- Rowbury, R. J., Hussain, N. H., and Goodson, M. (1998) Extracellular proteins and other components as obligate intermediates in the induction of a range of acid tolerance and sensitisation responses in *Escherichia coli*. *FEMS Microbiol. Lett.*, **166**, 283–288.
- Sakuma, T., Yamada, N., Saito, H., Kakegawa, T., and Kobayashi, H. (1998) pH dependence of the function of sodium ion extrusion systems in *Escherichia coli*. *Biochim. Biophys. Acta*, **1363**, 231–237.
- Sato, M., Machida, K., Arikado, E., Saito, H., Kakegawa, T., and Kobayashi, H. (2000) Expression of outer membrane proteins in *Escherichia coli* growing at acid pH. *Appl. Environ. Microbiol.*, **66**, 943–947.
- Shechter, E., Letellier, L., and Simons, E. R. (1982) Fluorescence dye as monitor of internal pH in *Escherichia coli* cells. *FEBS Lett.*, **139**, 121–124.
- Sjostrom, J. E. and Larsson, H. (1996) Factors affecting growth and antibiotic susceptibility of *Helicobacter pylori*: Effect of pH and urea on the survival of a wild-type strain and a urease-deficient mutant. *J. Med. Microbiol.*, **44**, 425–433.
- Slonczewski, J. L., Rosen, B. P., Alger, J. R., and Macnab, R. M. (1981) pH homeostasis in *Escherichia coli*: Measure-

- ment by ^{31}P nuclear magnetic resonance of methylphosphonate and phosphate. *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 6271–6275.
- Small, P., Blankenhorn, D., Welty, D., Zinser, E., and Slonczewski, J. L. (1994) Acid and base resistance in *Escherichia coli* and *Shigella flexneri*: Role of *rpoS* and growth pH. *J. Bacteriol.*, **176**, 1729–1737.
- Smith, A. J., Quivey, R. G., Jr., and Faustoferri, R. C. (1996) Cloning and nucleotide sequence analysis of the *Streptococcus mutans* membrane-bound, proton-translocating ATPase operon. *Gene*, **183**, 87–96.
- Smith, R. L., Kaczmarek, M. T., Kucharski, L. M., and Maguire, M. E. (1998) Magnesium transport in *Salmonella typhimurium*: Regulation of *mgtA* and *mgtCB* during invasion of epithelial and macrophage cells. *Microbiology*, **144**, 1835–1843.
- Stim, K. P. and Bennett, G. N. (1993) Nucleotide sequence of the *adi* gene, which encodes the biodegradative acid-induced arginine decarboxylase of *Escherichia coli*. *J. Bacteriol.*, **175**, 1221–1234.
- Suziedlien, E., Suziedlis, K., Garbencit, V., and Normark, S. (1999) The acid-inducible *asr* gene in *Escherichia coli*: Transcriptional control by the *phoBR* operon. *J. Bacteriol.*, **181**, 2084–2093.
- Suzuki, T. and Kobayashi, H. (1989) Regulation of the cytoplasmic pH by a proton-translocating ATPase in *Streptococcus faecalis* (faecium). A computer simulation. *Eur. J. Biochem.*, **180**, 467–471.
- Tabor, C. W. and Tabor, H. (1985) Polyamines in microorganisms. *Microbiol. Rev.*, **49**, 81–99.
- Taglicht, D., Padan, E., and Schuldiner, S. (1991) Overproduction and purification of a functional Na^+/H^+ antiporter coded by *nhaA* (*ant*) from *Escherichia coli*. *J. Biol. Chem.*, **266**, 11289–11294.
- Takayama, M., Ohya, T., Igarashi, K., and Kobayashi, H. (1994) *Escherichia coli cad* operon functions as a supplier of carbon dioxide. *Mol. Microbiol.*, **11**, 913–918.
- Thomas, A. D. and Booth, I. R. (1992) The regulation of expression of the porin gene *ompC* by acid pH. *J. Gen. Microbiol.*, **138**, 1829–1835.
- Trchounian, A. and Kobayashi, H. (1999) Kup is the major K^+ uptake system in *Escherichia coli* upon hyper-osmotic stress at a low pH. *FEBS Lett.*, **447**, 144–148.
- Watson, N., Dunyak, D. S., Rosey, E. L., Slonczewski, J. L., and Olson, E. R. (1992) Identification of elements involved in transcriptional regulation of the *Escherichia coli cad* operon by external pH. *J. Bacteriol.*, **174**, 530–540.
- Yamasaki, K., Moriyama, Y., Futai, M., and Tsuchiya, T. (1980) Uptake and extrusion of K^+ regulated by extracellular pH in *Escherichia coli*. *FEBS Lett.*, **120**, 125–127.