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Streptococcus faecalis Mutants Defective in Regulation of Cytoplasmic pH

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We have isolated two acid-sensitive mutants of *Streptococcus faecalis* (ATCC 9790), designated AS13 and AS25, which grew at pH 7.5 but not at pH below 6.0. The ionophore gramicidin D, which collapsed the pH gradient between the cytoplasm and the medium, had little effect on the growth of these mutants, indicating that growing cells maintain only a small pH gradient. In the presence of gramicidin D the growth rates of the parent and mutant strains were identical over a range of pH values. When glucose was added to a cell suspension at pH 6.4, the parent strain generated a pH gradient of 1.0 unit, interior alkaline; AS13 generated a pH gradient of only 0.5 units, and AS25 generated no measurable pH gradient. The proton permeability of the mutant strains was the same as that of the parent strain. These results suggest that a cytoplasmic pH of around 7.5 is required for the growth of the cells and that the mutant strains are unable to establish a neutral cytoplasmic pH in acidic medium because of damage to the regulatory system of the cytoplasmic pH. Mutant strains also have a reduced capacity to extrude protons and take up potassium. Therefore, it is likely that these cation transport systems are involved in the regulation of cytoplasmic pH.

During the past decade, various methods have been developed to measure the cytoplasmic pH of bacteria (6, 29). These revealed the interesting fact that the cytoplasmic pH is near neutrality in all bacteria studied, including acidophilic and alkalophilic ones (5, 7, 12, 16, 17, 19, 23, 24). Bacteria growing in neutral media should have no difficulty in maintaining the cytoplasm near neutrality, but those growing in acidic or alkaline media may require specific mechanisms. Two hypotheses have been proposed to account for the constancy of cytoplasmic pH. According to the first hypothesis, the cytoplasmic pH is stable and independent of cellular metabolism (5, 16, 23); the pH would be maintained by physicochemical mechanisms, for example, by a Donnan potential (16). According to the second hypothesis, the cytoplasmic pH is regulated by mechanisms that depend on metabolic energy (2, 3, 7-9, 12, 17, 24, 30, 31). The latter hypothesis required two mechanisms, one to raise the cytoplasmic pH and another to lower it. Harold and co-workers (8, 9) proposed that the cytoplasmic pH is raised by metabolic extrusion of protons and electrogenic influx of potassium. Lowering of the cytoplasmic pH could be accomplished by sodium proton antiporter (24, 30, 31) or by potassium proton antiporter (2, 3). In any event, the movement of monovalent cations, including protons, is thought to be involved in the regulation of cytoplasmic pH.

We thought that mutants defective in the regulation of cytoplasmic pH would be useful tools in the study of this problem, and we tried to isolate such mutants. *Streptococcus faecalis* was chosen for this study for the following reasons. *S. faecalis* grows over a wide range of pH values. Harold and co-workers (12, 14) showed that even cells growing in acidic medium maintained a neutral cytoplasmic pH and, further, that maintenance of such a pH gradient (interior alkaline) was essential for growth in acidic medium but not in neutral medium. This tells us that it should be possible to isolate mutants defective in the regulation of cytoplasmic pH by selecting strains that grow at neutral but not at acidic pH. Furthermore, much work has been done on the movement of cations across the cytoplasmic membrane of *S. faecalis* (9, 11, 14). This is important for the present work because the earlier work has suggested the involvement of cation transport in the regulation of cytoplasmic pH.

In this paper we describe the isolation of mutants of *S. faecalis* which grow at pH 7.5 but not at pH 6.0. Several observations suggest that the regulation of cytoplasmic pH is defective in these mutants.

MATERIALS AND METHODS

Organism and growth conditions. *S. faecalis* (ATCC 9790) was generously supplied by F. M. Har-

old. *S. faecalis* was grown at 37°C in a medium containing 20 g of K_2HPO_4, 10 g of tryptone (Difco Laboratories), 5 g of yeast extract (Difco), and 10 g of glucose per liter. The pH of the autoclaved medium was 7.5; it was adjusted by the addition of KOH or HCl where indicated. The growth rate is expressed as the growth rate constant, defined by the following formula (32): $\mu = (\ln N - \ln N_0)/(t - t_0)$, where the values of N and N_0 correspond to the number of bacteria in the culture at time t and t_0, respectively, and ln is the natural logarithm.

Chemicals. ^{14}C-labeled acetylsalicylic acid and ^{3}H-labeled sorbitol were obtained from New England Nuclear Corp. Gramicidin D and valinomycin were purchased from Sigma Chemical Co. Tetrachlorosalicylanilide was a gift from F. M. Harold. Tryptone, yeast extract, and SF medium were purchased from Difco. Other reagents used were of analytical grade.

Isolation of acid-sensitive mutants. *S. faecalis* was treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (300 μg/ml) in the growth medium (pH 7.5) for 1 h at 37°C. The mutants were allowed to segregate during 2 h of growth at pH 7.5 at 37°C; mutant cultures were then enriched by penicillin G (200 U/ml) in the growth medium (pH 6.0) at 37°C. Six colonies were picked up which grew on the plate at pH 7.5 but not at pH 6.0. All of the mutant strains grew on the plate of SF medium; all were catalase negative, contained no hemeproteins, and appeared identical to the parent strain under the microscope. We refer to these as acid-sensitive (AS) mutants; two strains, AS13 and AS25, were selected for this study. The reversion frequencies of AS13 and AS25 were 10^{-6} and 10^{-8}, respectively, indicating point mutation.

Measurement of the cytoplasmic volume. ^{3}H-labeled sorbitol (15.1 μCi/mmol, 0.2 μCi/ml) was added to the cell suspension (6 to 25 mg [dry weight] per ml), and the suspension was centrifuged with a microcentrifuge (5412; Eppendorf Co.) at maximal speed for 2 min. The volume of the interstitial water of the pellet was calculated from the content of ^{3}H-labeled sorbitol. The cytoplasmic volume was calculated from the water content (wet weight minus dry weight of the pellet) and the volume of the interstitial water of the pellet. The cytoplasmic volume was found to be 1.20 μl per mg (dry weight) of cells, a value identical to that reported by Harold and Spitz (13).

Measurements of the pH gradient and membrane potential by flow dialysis. The procedure of Ramos et al. (28) was employed at room temperature; 800 μl of cell suspension (50 mM potassium maleate–2 mM $MgSO_4$, 17 to 19 mg [dry weight] of cells per ml) were used in each measurement, and the flow rate was 2 ml/min. The pH gradient was calculated from the distribution of ^{14}C-labeled acetylsalicylic acid (33.4 mCi/mmol, 11.3 μM), which was not significantly metabolized under the conditions employed.

To measure the generation of membrane potential, cells were collected by centrifugation at the exponential phase of growth and washed with 2 mM $MgSO_4$. Washed cells were suspended in 50 mM tris(hydroxymethyl)aminomethane (Tris)-maleate-50 mM KCl–2 mM $MgSO_4$ (pH 7.5 or 6.5) at 20 to 22 mg (dry weight) per ml. The membrane potential was calculated from the distribution of ^{3}H-labeled triphenylmethylphosphonium (97.0 mCi/mmol, 60 μM), which was synthesized by the method of Hong (15). To calculate the pH gradient and membrane potential, the pK_a of acetylsalicylic acid was taken to be 3.49 (21), and the cytoplasmic volume was taken to be 1.20 μl per mg (dry weight) of cells.

Measurement of ion movements. Cells were collected by centrifugation at the exponential phase of growth and washed with 2 mM $MgSO_4$. Washed cells were suspended in 2 ml of 10 mM Tris-maleate–2 mM $MgSO_4$ (pH 6.4 or 7.7) at 1.2 to 1.5 mg of protein per ml. After addition of glucose (10 mM), changes in the pH of the cell suspension were monitored with the use of a pH electrode (Radiometer G2040C). The electrode was attached to a pH meter (Radiometer PHM64) connected to a recorder (Hitachi 065).

For measurement of the potassium movement, organisms were grown overnight in the medium which contained 8.5 g of Na_2HPO_4 per liter instead of K_2HPO_4. After being washed with 2 mM $MgSO_4$, cells were suspended in 20 ml of 10 mM Tris-maleate–2 mM KCl–2 mM $MgSO_4$ (pH 6.4 or 8.0) at 1.0 to 1.4 mg of protein per ml. After addition of glucose (10 mM), rates of the potassium accumulation were measured at room temperature with the use of a potassium electrode (Nishin Rika, Tokyo, Japan) attached to a Corning pH meter. Protein was determined by the method of Lowry et al. (20).

RESULTS

Growth of the mutant strains depends on the pH of the medium. At pH above 7 the growth rate of AS13 was the same as that of the parent strain. At pH 5.8 the parent strain grew almost as well as it did at pH 7.0, but AS13 grew very slowly (Fig. 1). When the pH was raised to

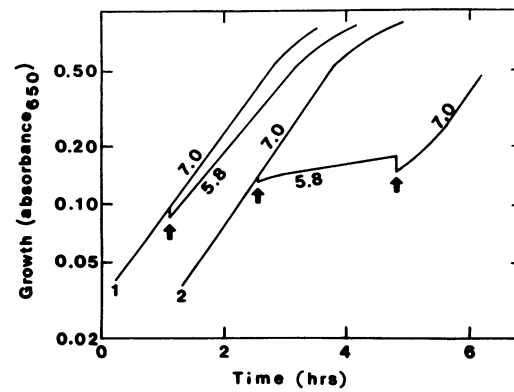

FIG. 1. *Growth of the parent and AS13 strains. Cells were grown as described in the text. Absorbance was measured with a Hitachi Spectrophotometer 181. The pH of the medium was occasionally adjusted to within 0.1 unit by the addition of 0.1 M KOH. The arrows indicate that the medium pH was changed by the addition of HCl or KOH. The decrease of the absorbance when pH was changed was due to the dilution of the medium by the addition of HCl or KOH. Line 1, parent; line 2, AS13.*

7.0 after 2 h at pH 5.8, AS13 resumed normal growth after a brief lag. The growth profile of AS25 was essentially the same as that of AS13, except that no growth was observed at pH 5.8, and the growth rate at pH 7.0 was slightly less than that of AS13. The growth rates of the parent strain, AS13, and AS25 were measured over a range of medium pH. As shown in Fig. 2, the growth rates of AS13 and AS25 fell sharply at pH below 7.0; AS25 was somewhat more acid sensitive than AS13. At pH 8.0 or above the growth rates of the parent and mutant strains were essentially identical.

Harold and Van Brunt (14) have reported that in rich medium containing a high concentration of potassium, gramicidin D collapsed the pH gradient and membrane potential and, consequently, that the cytoplasmic pH became equal to that of the medium. They also showed that growth under these conditions required that the pH of the medium be above 7 (14). The data shown in Fig. 3 confirm the drastic effect of gramicidin D on the growth of the parent strain at pH values below 7; they also document that gramicidin D has little effect on the growth rate of mutant strains AS13 and AS25. This suggests that gramicidin D induces only small changes in the cytoplasmic pH of mutant strains and implies that these mutants maintain only a small difference between the cytoplasmic pH and that of the medium. Figure 3 also shows that the growth rates of AS13 and AS25 in the presence of gramicidin D were identical to that of the parent strain over the entire range of medium pH. As mentioned above, a pH gradient is near zero in the presence of gramicidin D. These results therefore indicate that when the cytoplasmic pH is close to that of the medium, both parent and mutant strains grow equally well. It is indicated from these results that the mutation affects the regulation of the cytoplasmic pH.

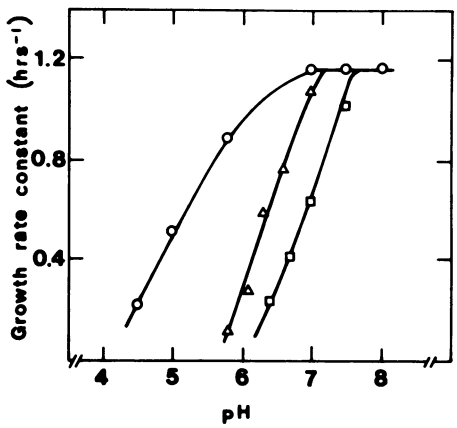

FIG. 2. *Effect of pH on the growth rates of the parent and mutant strains. The growth rates were measured at various pH of the medium as described in the text. Absorbance was measured with a Shimazu spectrophotometer at a concentration of less than 10^{-8} cells per ml because the change of the medium pH was negligible when the concentration of cells was less than this value. Symbols: (○) parent; (△) AS13; (□) AS25.*

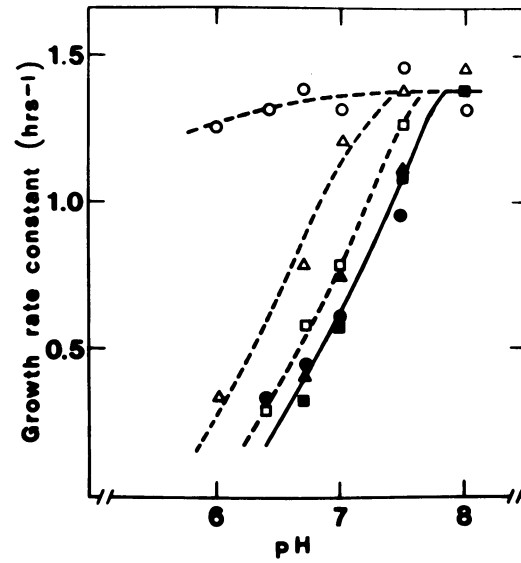

FIG. 3. *Effect of gramicidin D on the growth of the parent and mutant strains. Growth rates were measured as described in the legend to Fig. 2 in the presence (closed symbols) or absence (open symbols) of gramicidin D (4 μg/ml). Symbols: (●, ○) parent; (▲, △) AS13; (■, □) AS25.*

Generation of a pH gradient after addition of an energy source. In these experiments, generation of the pH gradient was measured by flow dialysis as described above. Organisms were suspended in 50 mM potassium maleate–2 mM $MgSO_4$ (pH 6.4); upon addition of glucose the parent strain generated a pH gradient of 1 unit, interior alkaline; the pH gradient was abolished upon the addition of tetrachlorosalicylanilide, a proton conductor (Fig. 4). Mutant AS13 generated a pH gradient of only 0.5 units, interior alkaline, under these conditions, and mutant AS25 generated no measurable pH gradient (Fig. 4). The difference between AS13 and AS25 is consistent with the fact that AS13 is somewhat less sensitive to acid pH than is AS25. These results indicate that the mutant strains are unable to generate a pH gradient sufficiently large to keep the cytoplasmic pH above 7.5.

Proton permeability of the cytoplasmic membrane in the mutant strains. From the preceding results it appears that mutant strains

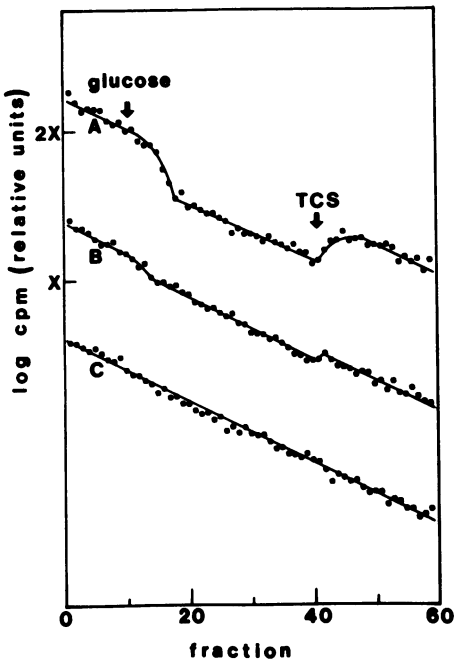

FIG. 4. *Generation of ΔpH in the parent and mutant strains. Cells grown overnight as described in the text were collected by centrifugation and washed with 10 mM potassium maleate-2 mM $MgSO_4$ (pH 6.4). Washed cells were suspended in 50 mM potassium maleate-2 mM $MgSO_4$ (pH 6.4). Flow dialysis was done as described in the text. Glucose (20 mM) and tetrachlorosalicylanilide (20 μM) were added where indicated. The radioactivity (approximately 7,000 cpm) obtained in the initial fraction was similar in all experiments. A, Parent (19 mg [dry weight] per ml); B, AS13 (17 mg [dry weight] per ml); C, AS25 (17 mg [dry weight] per ml).*

are unable to maintain the cytoplasmic pH at above 7.5 when the pH of the medium is acidic; in other words, the regulation of the cytoplasmic pH is defective in the mutant strains. However, one point still remains to be clarified. If the mutants had become leaky to protons they might have been unable to regulate the cytoplasmic pH even if the regulatory system itself was normal. We have therefore measured the proton permeability of both parent and mutant strains by monitoring the change of the medium pH after a proton pulse. Figure 5A documents that the proton permeabilities of both mutants AS13 and AS25 are very similar to that of the parent strain; in all cases an influx of protons was observed for 6 min or more after a proton pulse. The rate of proton influx was somewhat enhanced by the addition of valinomycin, but again there was no difference in the rates of proton uptake between parent and mutant strains (Fig. 5B). In the presence of gramicidin D the rate of proton influx was very rapid and attained equilibrium in as little as 1 min (Fig. 5C). The effect of tetrachlorosalicylanilide was the same as that of gramicidin D (data not shown).

By using the flow dialysis method, we also monitored the change in cytoplasmic pH in response to shift of the pH of the medium from 6.0 to 7.5. No significant difference between parent and mutant strains was observed in the absence of the energy source (data not shown). We therefore concluded that the failure of the mutant strains to regulate their cytoplasmic pH is not the result of an alteration of the passive proton permeability of the cytoplasmic membrane.

Movements of potassium and protons in the mutant strains. The rates of proton extrusion in AS13 and AS25 strains were remarkably

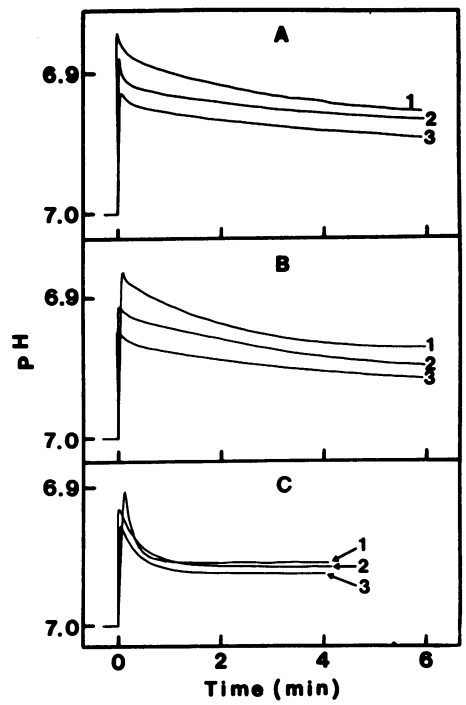

FIG. 5. *Proton influx after a proton pulse. Cells grown as described in the text were collected in the mid-log phase of growth, washed with 2 mM $MgSO_4$, and suspended in 10 mM KCl-2 mM $MgSO_4$. The change of the medium pH was measured as described in the text after the medium pH was adjusted to 7.0. Two microliters of 0.1 N HCl was added to the cell suspension at time zero. A, No addition; B, valinomycin (2 μg/ml); C, gramicidin D (4 μg/ml). Line 1, parent (17 mg [dry weight] per ml); line 2, AS13 (13 mg [dry weight] per ml); line 3, AS25 (13 mg [dry weight] per ml).*

reduced at both pH 6.4 and 7.7 in comparison with that of the parent strain (Table 1). Moreover, it was shown that the proton extrusion in mutant strains was not inhibited by dicyclohexylcarbodiimide, an inhibitor of a proton-translocating ATPase (Table 1). These results indicate that the activity of the proton extrusion through a proton-translocating ATPase is deficient in mutant AS13 and AS25. This conclusion was reinforced by the measurement of the generation of membrane potential. Upon addition of glucose the parent strain generated approximately 70 mV of the membrane potential at pH 7.6 and 6.4 in the presence of 50 mM KCl. By contrast, no measurable membrane potential was generated in either strain AS13 or AS25 under the same conditions. The value of the membrane potential generated in the parent strain was identical to that reported by Bakker and Harold (1).

The uptake of potassium ions after the addition of glucose was measured as described above. The rates of potassium accumulation in the parent strain were 110 and 190 nmol/min per mg of protein at pH 6.4 and 8.0, respectively. By contrast, the rate of potassium accumulation was 20 nmol/min per mg of protein at pH 8.0, and no measurable accumulation was observed at pH 6.4 in either strain AS13 or AS25 under the same conditions. These results indicate that potassium accumulation was also deficient in mutant strains. The results obtained here strongly support the hypothesis proposed by Harold and coworkers (8, 9) that movements of protons and potassium are involved in the regulation of the cytoplasmic pH.

Although mutant AS13 was less sensitive to acid pH than AS25 and AS13 could generate a small pH gradient, no significant difference was observed between AS13 and AS25 in proton extrusion and potassium accumulation. This might be due to the limited sensitivities of the methods used here. Indeed, it was difficult to detect proton extrusion and potassium accumulation amounting to less than 10 nmol/min per mg of protein.

TABLE 1. *Proton extrusion in the parent and mutant strains*[a]

Strain	Inhibitor[b]	Proton extrusion (μmol/min/mg of protein)	
		pH 6.4	pH 7.7
Parent	None	0.28	0.21
	DCCD	0.02	0.09
AS13	None	0.02	0.10
	DCCD	0.02	0.09
AS25	None	0.02	0.10
	DCCD	0.03	0.09

[a] Proton extrusion was measured at 28°C as described in the text. The changes of 0.01 pH units at pH 6.4 and 7.7 corresponded to the proton extrusion of 39 and 7.4 nmol of protons per ml, respectively.

[b] Cells were treated with 10^{-4} M dicyclohexylcarbodiimide (DCCD) at room temperature for over 15 min in the reaction buffer.

DISCUSSION

The method used here for the isolation of mutants should in principle allow the isolation of several types of pH-sensitive mutants. For example, Colb and Shapiro (4) isolated a pH-sensitive mutant of *Escherichia coli* which could not grow on lactose medium at pH 8.1; they concluded that the pH sensitivity is due to a change in pH dependence of β-galactosidase, whereas the cytoplasmic pH itself is normal. We described the isolation of acid-sensitive mutants AS13 and AS25 which appear to be defective in the regulation of the cytoplasmic pH. This conclusion is based on the following findings. (i) Gramicidin D had little effect on the growth of these mutants, indicating that growing cells maintained only a small pH gradient. (ii) Under conditions such that the cytoplasmic pH was close to that of the medium, the parent and mutant strains grew at similar rates over a wide pH range. (iii) When glucose was added to a cell suspension at pH 6.4, the parent strain generated a pH gradient of 1 unit, interior alkaline. Under these conditions, AS13 could generate a pH gradient of only 0.5 units and AS25 generated no pH gradient at all. (iv) The proton permeability of the mutant strains was the same as that of the parent strain.

At the beginning of this study we were not certain whether the cytoplasmic pH was controlled by a regulatory system dependent on the metabolic energy. Some investigators have reported that the cytoplasmic pH is stable and does not depend on energy metabolism (5, 16, 23). On the other hand, there have been several reports describing the effects of inhibitors of energy metabolism on the cytoplasmic pH of bacteria (7, 12, 17, 24). We succeeded in isolating mutants which are defective in the regulation of cytoplasmic pH; this strongly suggests that *S. faecalis* has a system that regulates cytoplasmic pH.

According to chemiosmotic theory, bacteria generate a pH gradient and membrane potential as part of the mechanisms that synthesize ATP during oxidative phosphorylation and drive the accumulation of amino acids, sugars, and cations (8, 22). We report here that acid-sensitive mutants were unable to generate a pH gradient

sufficiently large to keep the cytoplasmic pH at 7.5, resulting in a reduction of the growth rate when pH of the medium is below 7.5. This seems to us very important, for it suggests that the generation of the pH gradient is required for the growth, not so much to drive transport systems as to regulate the cytoplasmic pH, particularly when cells are growing at pH below 7.5. Raven and Smith (26) have argued that the primitive heterotroph used the proton-translocating ATPase for the extrusion of protons, rather than to produce ATP.

Ramos and co-workers (27, 28) measured the magnitude of the pH gradient and membrane potential generated by membrane vesicles of *E. coli* suspended in buffers of varying pH. They reported the interesting fact that the internal pH of the membrane vesicles was always much the same regardless of the pH of the medium (27, 28). This suggests that the regulatory system of the cytoplasmic pH is localized at the cytoplasmic membrane and can function even in membrane vesicles. It seems likely that bacteria require two distinct systems to regulate the cytoplasmic pH, one to raise the pH and the other to lower it. Harold and co-workers (8, 9) proposed that movements of protons and potassium are involved in the system that elevates the cytoplasmic pH. The activities of proton extrusion and potassium uptake were greatly reduced in our acid-sensitive mutants. To this extent the phenotype of acid-sensitive mutants resembles that of a mutant defective in the retention of potassium ion originally isolated by Harold et al. (10, 11). It is still not entirely clear what is wrong with that mutant, but Harold et al. (1, 11) reported that this mutant is defective in the generation of proton motive force and has a reduced ability to accumulate potassium. It is suggested that the defect in our acid-sensitive mutants also involves the metabolic extrusion of protons through proton-translocating ATPase. ATPase activities in AS13 and AS25 were 44 and 30%, respectively, of that in the parent strain. It seems to us that these values are still too high to explain the reduction of proton extrusion, especially in AS25, and it is therefore possible that the F_0 portion of ATPase is defective. Studies on this point are in progress. We would point out here that our mutant strains differ somewhat from those described by Harold and co-workers. Their mutant strain was able to grow at pH 6 when supplied with a high concentration of potassium ion. By contrast, AS25 was unable to grow at pH 6 in our medium, which contained approximately 0.1 M K_2HPO_4 (Fig. 2).

Krulwich et al. (7, 18) showed that the cytoplasmic pH of alkalophilic bacteria is lower than that of the medium, and they attribute this to the operation of a sodium proton antiporter. The cytoplasmic pH of *E. coli* was shown to be slightly acidic when the pH of the medium was above 7.5 (24, 33), and it was proposed that the cytoplasmic pH is acidified by a sodium proton antiporter (24, 30, 31) or a potassium proton antiporter (2, 3). Plack and Rosen (25) recently isolated a mutant of *E. coli* which was unable to grow at alkaline pH, and they found that the potassium proton exchange activity was defective in their mutant. The growth rate of *S. faecalis* was markedly reduced when the medium pH was increased above 8, and growth was very slow at pH 9 (unpublished data). The growth rate of our mutant strains was the same as that of the parent strain at pH above 8. Furthermore, the cytoplasmic pH of *S. faecalis* was acid when cells were grown in the presence of gramicidin D (ΔpH, approximately 0.4 units; interior acid; unpublished data), and it appears that this is the result of a Donnan potential. Thus, a small pH gradient, interior acid, can be generated by a Donnan potential. It therefore appears doubtful that *S. faecalis* has a system capable of lowering the cytoplasmic pH.

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LITERATURE CITED

1. **Bakker, E. P., and F. M. Harold.** 1980. Energy coupling to potassium transport in *Streptococcus faecalis*: interplay of ATP and the protonmotive force. J. Biol. Chem. **255**:433–440.
2. **Beck, J. C., and B. P. Rosen.** 1979. Cation/proton antiport systems in *Escherichia coli*: properties of the sodium/proton antiporter. Arch. Biochem. Biophys. **194**: 208–214.
3. **Brey, R. N., B. P. Rosen, and E. N. Sorensen.** 1980. Cation/proton antiport systems in *Escherichia coli*: properties of the potassium/proton antiporter. J. Biol. Chem. **255**:39–44.
4. **Colb, M., and L. Shapiro.** 1977. A pH-conditional mutant of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **74**: 5637–5641.
5. **Cox, J. C., D. G. Nicholls, and W. J. Ingledew.** 1979. Transmembrane electrical potential and transmembrane pH gradient in the acidophile *Thiobacillus ferrooxidans*. Biochem. J. **178**:195–200.
6. **Gillies, R. J., and D. W. Deamer.** 1979. Intracellular pH: methods and applications. Curr. Top. Bioenerg. **9**: 63–87.
7. **Guffanti, A. A., R. Blanco, and T. A. Krulwich.** 1979. A requirement for ATP for β-galactoside transport by *Bacillus alcalophilus*. J. Biol. Chem. **254**:1033–1037.
8. **Harold, F. M.** 1977. Ion currents and physiological functions in microorganisms. Annu. Rev. Microbiol. **31**:181–203.
9. **Harold, F. M., and K. Altendorf.** 1974. Cation transport in bacteria: K^+, Na^+, and H^+. Curr. Top. Membr. Transp. **5**:1–50.
10. **Harold, F. M., R. L. Harold, J. R. Baarda, and A.**

Abrams. 1967. A genetic defect in retention of potassium by *Streptococcus faecalis*. Biochemistry **6**:1777–1784.

11. **Harold, F. M., and D. Papineau.** 1972. Cation transport and electrogenesis by *Streptococcus faecalis*. II. Proton and sodium extrusion. J. Membrane Biol. **8**:45–62.
12. **Harold, F. M., E. Pavlasova, and J. R. Baarda.** 1970. A transmembrane pH gradient in *Streptococcus faecalis*: origin, and dissipation by proton conductors and N,-N'-dicyclohexylcarbodiimide. Biochim. Biophys. Acta **196**:235–244.
13. **Harold, F. M., and E. Spitz.** 1975. Accumulation of arsenate, phosphate, and aspartate by *Streptococcus faecalis*. J. Bacteriol. **122**:266–277.
14. **Harold, F. M., and J. Van Brunt.** 1977. Circulation of H^+ and K^+ across the plasma membrane is not obligatory for bacterial growth. Science **197**:372–373.
15. **Hong, J.** 1977. An *ecf* mutation in *Escherichia coli* pleiotropically affecting energy coupling in active transport but not generation or maintenance of membrane potential. J. Biol. Chem. **252**:8582–8588.
16. **Hsung, J. C., and A. Haug.** 1975. Intracellular pH of *Thermoplasma acidophila*. Biochim. Biophys. Acta **389**:477–482.
17. **Krulwich, T. A., L. F. Davidson, S. J. Filip, Jr., R. S. Zuckerman, and A. A. Guffanti.** 1978. The protonmotive force and β-galactoside transport in *Bacillus acidocaldarius*. J. Biol. Chem. **253**:4599–4603.
18. **Krulwich, T. A., K. G. Mandel, R. F. Bornstein, and A. A. Guffanti.** 1979. A non-alkalophilic mutant of *Bacillus* alkalophilus lacks the Na^+/H^+ antiporter. Biochem. Biophys. Res. Commun. **91**:58–62.
19. **Langworthy, T. A.** 1978. Microbial life in extreme pH values, p. 279–315. *In* D. J. Kushner (ed.), Microbial life in extreme environments. Academic Press, Inc., London.
20. **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.
21. **Maloney, P. C.** 1977. Obligatory coupling between proton entry and the synthesis of adenosine 5'-triphosphate in *Streptococcus lactis*. J. Bacteriol. **132**:564–575.
22. **Mitchell, P.** 1976. Vectorial chemistry and the molecular mechanics of chemiosmotic coupling: power transmission by proticity. Biochem. Soc. Trans. **4**:399–430.
23. **Oshima, T., H. Arakawa, and M. Baba.** 1977. Biochemical studies on an acidophilic, thermophilic bacterium, *Bacillus acidocaldarius*: isolation of bacteria, intracellular pH, and stabilities of biopolymers. J. Biochem. **81**: 1107–1113.
24. **Padan, E., D. Zilberstein, and H. Rottenberg.** 1976. The proton electrochemical gradient in *Escherichia coli* cells. Eur. J. Biochem. **63**:533–541.
25. **Plack, H. R., Jr., and B. P. Rosen.** 1980. Cation/proton antiport systems in *Escherichia coli*. Absence of potassium/proton antiporter activity in a pH-sensitive mutant. J. Biol. Chem. **255**:3824–3825.
26. **Raven, J. A., and F. A. Smith.** 1976. The evolution of chemiosmotic energy coupling. J. Theor. Biol. **57**:301–312.
27. **Ramos, S., and H. R. Kaback.** 1977. The electrochemical proton gradient in *Escherichia coli* membrane vesicles. Biochemistry **16**:848–854.
28. **Ramos, S., S. Schuldiner, and H. R. Kaback.** 1976. The electrochemical gradient of protons and its relationship to active transport in *Escherichia coli* membrane vesicles. Proc. Natl. Acad. Sci. U.S.A. **73**:1892–1896.
29. **Rottenberg, H.** 1975. The measurement of transmembrane electrochemical proton gradients. J. Bioenerg. **7**: 61–74.
30. **Schuldiner, S., and H. Fishkes.** 1978. Sodium-proton antiport in isolated membrane vesicles of *Escherichia coli*. Biochemistry **17**:706–711.
31. **Skulachev, V. P.** 1978. Membrane-linked energy buffering as the biological function of Na^+/K^+ gradient. FEBS Lett. **87**:171–179.
32. **Stanier, R. Y., E. A. Adelberg, J. L. Ingraham, and M. L. Wheelis.** 1979. Introduction to the microbial world. Prentice-Hall, Inc., Englewood Cliffs, N.J.
33. **Zilberstein, D., S. Schuldiner, and E. Padan.** 1979. Proton electrochemical gradient in *Escherichia coli* cells and its relation to active transport of lactose. Biochemistry **18**:669–673.