JOURNAL OF BACTERIOLOGY, May 1982, p. 506–511
0021-9193/82/050506-06$02.00/0

Vol. 150, No. 2

Second System for Potassium Transport in *Streptococcus faecalis*

HIROSHI KOBAYASHI

Department of Membrane Biochemistry, Research Institute for Chemobiodynamics, Chiba University, 1-8-1, Inohana, Chiba, 280, Japan

Received 29 September 1981/Accepted 12 December 1981

It has been reported that the accumulation of K^+ by *Streptococcus faecalis* is mediated by a transport system which requires both ATP and the proton motive force (Bakker and Harold, J. Biol. Chem. **255**:433–440, 1980). My results indicate that *S. faecalis* has a second transport system for K^+. The features of this system are as follows: (i) the system is driven by ATP (or a derivative of ATP) and does not require the proton motive force; (ii) the system is normally absent in the wild-type strain but can be derepressed by lowering the intracellular concentration of K^+; (iii) the pH optimum of this system is about 8.5, and no detectable K^+ is accumulated at pH values below 6.5; and (iv) the rate of Rb^+ accumulation by this system is very low. These properties are quite different from those of the transport system described by Bakker and Harold. Therefore, I propose that *S. faecalis* has two K^+ transport systems.

Since the potassium ion is an essential cation for bacterial growth, the transport system of this ion has been studied extensively (4, 5). In *Escherichia coli*, it is clear that the accumulation of K^+ is mediated largely by two systems, designated Kdp and TrkA (2). The Kdp system is driven by ATP, and the TrkA system requires both ATP and the proton motive force (12). The physiological functions of the K^+ transport system are also interesting. In addition to maintaining the intracellular level of K^+ required to activate protein synthesis and metabolic enzymes, K^+ transport plays an essential role in the regulation of cytoplasmic pH and osmolarity in bacteria (2, 4). In this line of inquiry, interesting findings have also been made with *Streptococcus faecalis* (4, 5, 9), but the K^+ transport systems of *S. faecalis* are not as well defined as those of *E. coli*.

Recently, Bakker and Harold (1) reported that in *S. faecalis* K^+ accumulation is mediated by a transport system which, like the TrkA system of *E. coli*, requires both ATP and the proton motive force. Unemoto and I reported previously that mutants of *S. faecalis* defective in the regulation of cytoplasmic pH were unable to generate the proton motive force (9). Therefore, I expected that such mutants would require media containing high K^+ concentrations for growth. Surprisingly, such mutants could grow in medium containing less than 10 mM K^+ (unpublished data). This finding suggested that *S. faecalis* must be able to accumulate K^+ in the absence of the proton motive force. In this paper, I describe a second *S. faecalis* K^+ transport system that does not require the proton motive force and resembles the Kdp system of *E. coli*.

MATERIALS AND METHODS

Organisms and growth conditions. *S. faecalis* ATCC 9790 was generously supplied by F. M. Harold. Mutant AS25 was derived from *S. faecalis* as described previously (9). Bacteria were grown at 37°C on the following complex media. Medium 2KTY contained (per liter) 20 g of K_2HPO_4, 10 g of tryptone, 5 g of yeast extract, and 10 g of glucose; the final K^+ concentration was approximately 0.2 M. Medium NaTY was the same as medium 2KTY, except that 8.5 g of Na_2HPO_4 was used instead of K_2HPO_4; medium NaTY contained 5 to 10 mM K^+. To prepare arginine-adapted cells, bacteria were grown on medium containing 1.0 g of galactose per liter and 10 g of arginine per liter instead of glucose. All media were adjusted to pH 7.5 to 8.0.

Chemicals. Monactin and tetrachlorosalicylanilide (TCS) were gifts from F. M. Harold. Tryptone and yeast extract were purchased from Difco Laboratories. Carbonyl cyanide *m*-chlorophenyl hydrazone was purchased from Sigma Chemical Co. Erythromycin was a gift from S. Hirose. Chloramphenicol was purchased from Boehringer Mannheim GmbH. Other reagents were of analytical grade.

Measurement of K^+ movements. (i) Filtration method. Cells grown overnight were washed twice with 2 mM $MgSO_4$ and suspended in 20 ml of buffer; usually the buffer was 50 mM Na^+-HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid]–2 mM $MgSO_4$ (pH 8.2). Potassium chloride was added to a final concentration of 2 mM. During incubation at 25°C, 1-ml samples of the reaction mixture were filtered through polycarbonate membrane filters (Nuclepore Corp.); each filter was immersed in 3 ml of 5%

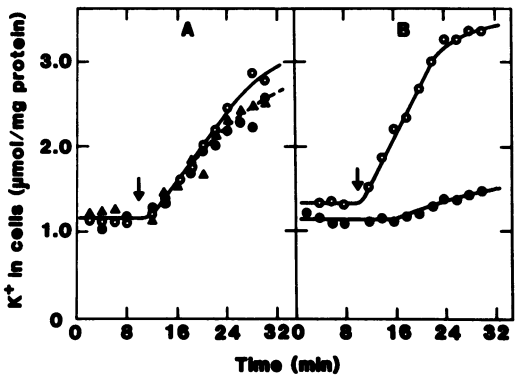

FIG. 1. Accumulation of K^+ by wild-type strain 9790 and strain AS25. Washed cells grown on medium NaTY were suspended in buffer (50 mM Na^+-HEPES–2 mM $MgSO_4$, pH 8.2), and the movement of K^+ was measured by the filtration method, as described in the text. Glucose (10 mM) was added at 10 min. TCS (10 μM) was added at zero time, and DCCD (0.1 mM) was added at −15 min. (A) Strain AS25 (0.18 mg of cell protein per ml). (B) Strain 9790 (0.23 mg of cell protein per ml). Symbols: ○, glucose only; ●, glucose plus TCS; ▲, glucose plus DCCD.

trichloroacetic acid and boiled for 5 min. The K^+ content of the extract was measured with an atomic absorption spectrophotometer (model 403; Hitach Perkin-Elmer) after centrifugation at 3,000 × *g* for 5 min.

(ii) Electrode method. Cells prepared as described above were suspended in 2 ml of buffer (usually 50 mM Na^+-HEPES–2 mM $MgSO_4$, pH 8.2), and potassium chloride was added to a final concentration of 2 mM. After glucose was added to a final concentration of 10 mM, the decrease in the K^+ content of the extracellular fluid was monitored at 25°C with a K^+ electrode (Radiometer A/S; model F2312K). A double-junction electrode (Radiometer A/S; model K701) filled with saturated KCl and 4 M lithium trichloroacetate solutions was used as a reference electrode. Both electrodes were attached to a pH meter (Radiometer A/S; model PHM64), which was connected to a recorder (Hitach 056). The amount of K^+ accumulated by the cells was calculated from the decrease in the extracellular K^+ concentration. Some experiments were conducted on a pH stat, using Tris as a titrant to maintain a constant pH. Rb^+ uptake was measured by the same procedure, except that 2 mM RbCl was used in place of KCl.

Preparation of K^+-depleted cells. The procedure of Harold and Baarda (6) was used. Cells were suspended in buffer (50 mM sodium maleate–2 mM $MgSO_4$, pH 8.0) at a concentration of approximately 2 mg of cell protein per ml, and monactin was added to a final concentration of 2 μg/ml. After the mixture was incubated at 25°C for 20 min, the cells were collected on a membrane filter (type HAWP; Millipore Corp.) and washed repeatedly with the same buffer.

Derepression of the Kdp system in the wild-type strain. Cells grown on medium NaTY were harvested during the logarithmic phase of growth and washed twice with 2 mM $MgSO_4$. The washed cells were suspended at a density of approximately 0.1 mg of cell protein per ml in medium containing (per liter) 0.85 g of Na_2HPO_4, 15 g of tryptone, 7.5 g of yeast extract, and 10 g of glucose. After monactin was added to a final concentration of 2 μg/ml, the cells were incubated at 37°C for 90 min on a pH stat, using NaOH as a titrant to maintain the pH at 8.0. During this treatment, both cell protein and absorbance at 650 nm increased approximately twofold. The cells were then collected by filtration through a Millipore filter (type HAWP) and washed repeatedly with buffer (50 mM sodium maleate–2 mM $MgSO_4$, pH 8.0).

Preparation of DCCD-treated cells. *N,N'*-Dicyclohexylcarbodiimide (DCCD) was added to a cell suspension to a final concentration of 0.1 mM. After the mixture was incubated at 25°C for 20 min, the cells were washed twice with 2 mM $MgSO_4$.Protein was determined by the method of Lowry et al. (10).

RESULTS

K^+ accumulation in a mutant defective in generation of the proton motive force. This study began with the observation that mutants defective in the generation of the proton motive force can still take up K^+. Cells of strain AS25 (one mutant belonging to this class) were grown overnight on medium NaTY and washed twice with 2 mM $MgSO_4$. The resulting washed cells contained 1.0 to 1.5 μmol of K^+ per mg of cell protein. Figure 1A shows that when glucose was added, K^+ was accumulated by strain AS25. As mentioned above, this strain cannot generate the proton motive force (9). As reported by Bakker and Harold (1), K^+ accumulation in the wild-type strain was strongly inhibited by the proton conductor TCS (Fig. 1B). By contrast, K^+ accu-

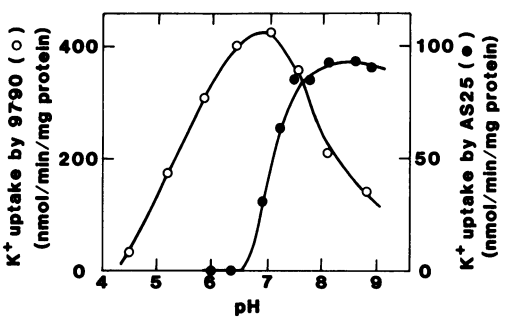

FIG. 2. Effect of pH on K^+ accumulation by wild-type strain 9790 and strain AS25. Washed cells grown on medium NaTY were suspended in buffer (50 mM Na^+-HEPES–2 mM $MgSO_4$, pH 8.2). After the pH of the assay mixture was adjusted with HCl or NaOH, K^+ uptake was assayed by the electrode method on a pH stat, as described in the text. Symbols: ○, strain 9790 (0.22 mg of cell protein per ml); ●, strain AS25 (0.24 mg of cell protein per ml).

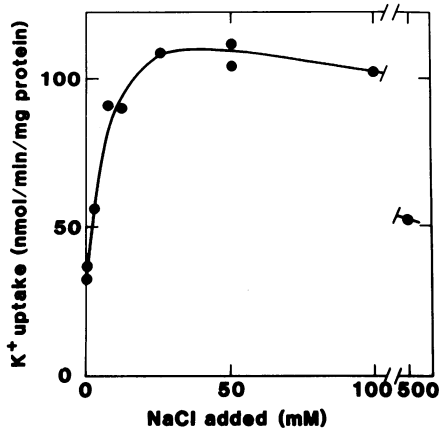

FIG. 3. Effect of NaCl on K^+ accumulation by strain AS25. Washed cells grown on medium NaTY were suspended in 2 mM $MgSO_4$ (0.21 mg of cell protein per ml). After NaCl was added, K^+ uptake was assayed at pH 8.2 by the electrode method on a pH stat, as described in the text. Before NaCl was added, the reaction mixture contained approximately 0.3 mM contaminating Na^+.

mulation by strain AS25 was not inhibited by TCS or by DCCD, an inhibitor of the H^+-translocating adenosine triphosphatase (H^+-ATPase) (Fig. 1A). The effect of carbonyl cyanide *m*-chlorophenyl hydrazone on K^+ accumulation was the same as that of TCS. In *S. faecalis*, the proton motive force is generated only by H^+-ATPase (7). Therefore, these results indicated that strain AS25 was able to accumulate K^+ in the absence of the proton motive force, whereas K^+ accumulation by the wild-type strain was completely dependent on this force.

To investigate the energy source for K^+ accumulation in strain AS25, the efficacy of arginine was compared with the efficacy of glucose. After arginine was added, K^+ was accumulated at a rate of 73.1 nmol/min per mg of cell protein. For comparison, the rate of K^+ accumulation when glucose was added was 84.8 nmol/min per mg of cell protein. In *S. faecalis*, ATP is only the product common to the metabolism of glucose and the metabolism of arginine. Therefore, I concluded that K^+ accumulation by strain AS25 was driven by ATP or one of its derivatives.

Properties of the K^+ transport system in strain AS25. In strain AS25 the rate of K^+ accumulation was maximal when the pH of the assay mixture was about 8.5; no detectable accumulation was observed at pH values below 6.5 (Fig. 2). The rates at pH values above 9 were not measured. By contrast, the maximum rate in the wild-type strain was at a pH of about 7.0 (Fig. 2).

In the wild-type strain of *S. faecalis*, Na^+ had essentially no effect on K^+ accumulation, as reported by Bakker and Harold (1). By contrast, K^+ accumulation by strain AS25 was stimulated markedly by Na^+ (Fig. 3). Stimulation of K^+ transport by Na^+ has also been reported in *Streptococcus lactis* (8) and *E. coli* (14).

In strain AS25, the rate of Rb^+ accumulation was about one-tenth the rate of K^+ accumulation, whereas in the wild-type strain it was near 80% of the rate of K^+ accumulation (Fig. 4). In *E. coli*, Rb^+ was accumulated by the TrkA system but not by the Kdp system (11, 13).

In strain AS25, the curve for K^+ accumulation was sigmoidal (Fig. 4). Therefore, I calculated a K_m value from the rates at 3 min after glucose was added, because the rate was almost linear at around 3 min and the intracellular concentration of K^+ was still low at that time. The K_m of the K^+ transport system of strain AS25 obtained from this calculation was 0.4 to 0.6 mM at pH 8.2. This value is very close to the values reported by Bakker and Harold (1) for the wild-type strain (0.20 mM at pH 7.3 and 0.12 mM at pH 8.0).

Next, I observed K^+ accumulation in cells grown on medium containing a high concentration of K^+. Whereas cells grown on medium NaTY (containing less than 10 mM K^+) were able to accumulate K^+, the rate of K^+ accumulation by cells grown on medium 2KTY (containing approximately 0.2 M K^+) was almost zero (Fig. 5). The same result was obtained with cells

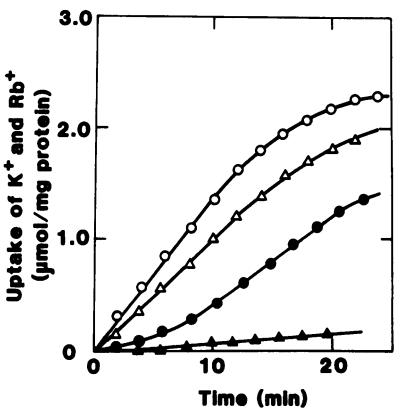

FIG. 4. Accumulation of K^+ and Rb^+ by wild-type strain 9790 and strain AS25. Cells were grown on medium NaTY, and intracellular K^+ was depleted as described in the text. Depleted cells were suspended in buffer (50 mM Na^+-HEPES–2 mM $MgSO_4$, pH 8.2) at a concentration of 0.21 mg of cell protein per ml, and the uptake of K^+ and the uptake of Rb^+ were assayed by the electrode method, as described in the text. Symbols: ○, K^+ uptake by strain 9790; △, Rb^+ uptake by strain 9790; ●, K^+ uptake by strain AS25; ▲, Rb^+ uptake by strain AS25.

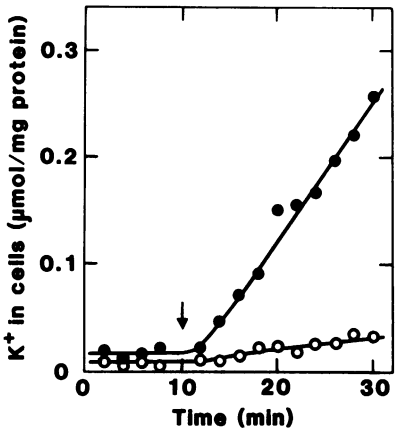

FIG. 5. Effect of growth on different media on K^+ accumulation by strain AS25. After the depletion of intracellular K^+, cells were suspended in buffer (50 mM Na^+-HEPES–2 mM $MgSO_4$, pH 8.2), and the movement of K^+ was measured by the filtration method, as described in the text. Glucose (10 mM) was added at 10 min. Symbols: ●, cells grown on medium NaTY (0.14 mg of cell protein per ml); ○, cells grown on medium 2KTY (0.20 mg of cell protein per ml).

harvested during the logarithmic phase of growth. These results suggest that the expression of this transport system is repressed when the intracellular level of K^+ is high.

Thus, the properties of the K^+ transport system in strain AS25 were quite different from those of the transport system in the wild-type strain, indicating that accumulation of K^+ in these two strains was mediated by different systems. As mentioned above, the transport system observed in strain AS25 was very similar to the Kdp system of *E. coli*. By contrast, the transport system observed in the wild-type strain was similar to the TrkA system of *E. coli* (1). By analogy with *E. coli*, I refer to these two *S. faecalis* systems as Kdp and Trk.

Kdp system in the wild-type strain. It is possible that the Kdp system observed in strain AS25 appeared because of a mutation in the gene(s) that specifies the K^+ transport system. However, this is unlikely. It has been suggested that the lesion in strain AS25 occurs in a gene for the H^+-ATPase and is a point mutation (9). In revertants of strain AS25, the characteristics of the K^+ transport system were essentially identical to those of the transport system in the wild-type strain (data not shown), even though there were many differences between K^+ transport in strain AS25 and K^+ transport in the wild-type strain. The most likely explanation is that the Kdp system is not expressed in the wild-type strain under normal growth conditions because intracellular K^+ is maintained at a high concentration by the Trk system. In strain AS25, the Trk system is not able to function because of the absence of the proton motive force, and consequently the Kdp system is expressed in strain AS25 grown on medium NaTY.

I next tried to derepress the Kdp system in the wild-type strain. Monactin is an ionophore that exchanges Na^+ for H^+ or K^+ for H^+ and is easily removed from cells by repeated washings. Thus, to derepress the Kdp system, the intracellular concentration of K^+ was lowered by adding monactin to growing cells of the wild-type strain; the cells were then washed repeatedly as described above. As reported by Bakker and Harold (1), K^+ accumulation in the wild-type strain was strongly inhibited by DCCD (Fig. 6A). By contrast, cells treated with monactin in the growth medium accumulated K^+ even when generation of the proton motive force was inhibited by DCCD (Fig. 6B). As mentioned above, in *S. faecalis* the proton motive force is generated solely by the H^+-ATPase (7). The properties of this DCCD-insensitive accumulation of K^+ in the wild-type strain were as follows: (i) the accumulation of K^+ was stimulated by Na^+ (Fig. 7A); (ii) no detectable accumulation of Rb^+ was observed (Fig. 7B); and (iii) the rate of K^+

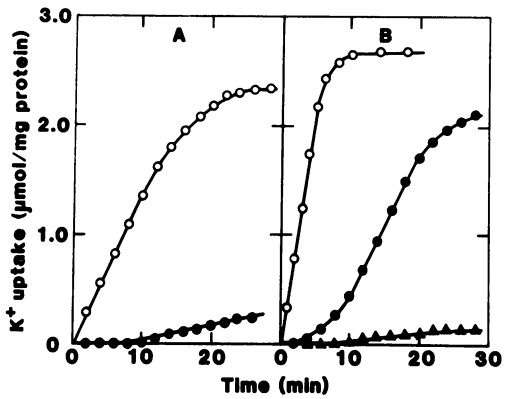

FIG. 6. Derepression of the Kdp system in the wild-type strain. Cells prepared as described below were suspended in buffer (50 mM Na^+-HEPES–2 mM $MgSO_4$, pH 8.2), and K^+ uptake was assayed by the electrode method, as described in the text. For the assay at pH 6.4, HCl was added to the cell suspension to adjust the pH. DCCD-treated cells were prepared as described in the text. (A) Normal cells. Cells were grown on medium NaTY, and internal K^+ was depleted as described in the text. Symbols: ○, control cells (0.21 mg of cell protein per ml), pH 8.2; ●, DCCD-treated cells (0.20 mg of cell protein per ml), pH 8.2. (B) Derepressed cells prepared as described in the text. Symbols: ○, control cells (0.30 mg of cell protein per ml), pH 8.2; ●, DCCD-treated cells (0.24 mg of cell protein per ml), pH 8.2; ▲, DCCD-treated cells (0.24 mg of cell protein per ml), pH 6.4.

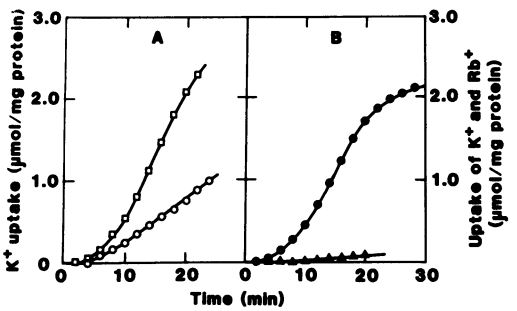

FIG. 7. Some properties of the Kdp system in the wild-type strain. Derepressed cells were prepared and treated with DCCD as described in the legend to Fig. 6. (A) Cells were suspended in 2 mM $MgSO_4$ at a concentration of 0.16 mg of cell protein per ml, and K^+ uptake was assayed at pH 8.2 as described in the legend to Fig. 3. Symbols: ○, no addition; □, 50 mM NaCl added. (B) Cells were suspended in buffer (50 mM Na^+-HEPES–2 mM $MgSO_4$, pH 8.2) at a concentration of 0.24 mg of cell protein per ml. The uptake of K^+ (●) and the uptake of Rb^+ (▲) were assayed by the electrode method, as described in the text.

uptake was very low at pH 6.4 (Fig. 6B). These properties were the same as those of K^+ accumulation in strain AS25. Therefore, I concluded that this DCCD-insensitive accumulation of K^+ was mediated by the Kdp system first observed in strain AS25.

When cells of the wild-type strain were treated with monactin in medium containing neither tryptone nor yeast extract, the activity of the Kdp system was very low (Fig. 8, line C). Moreover, the activity of the Kdp system decreased when cells were exposed to monactin in the presence of erythromycin (Fig. 8, line B). It should be noted that even DCCD-treated cells prepared from the wild-type strain grown normally did accumulate some K^+, although the rate was very low (Fig. 6). Judging from its pH dependence, this accumulation of K^+ was mediated by the Trk system (data not shown). Therefore, in Fig. 8 the net accumulation of K^+ mediated by the Kdp system can be expressed by the following relationship: line A (or line B or C) minus line D. Taking this correction into account, the activity of the Kdp system in cells treated with monactin in the presence of erythromycin was reduced to approximately 30% of its maximum value. Similar results were obtained when chloramphenicol was used instead of erythromycin (data not shown). These experiments with inhibitors of protein synthesis were conducted in rich medium, and this seems to be the reason why the expression of the Kdp system was not completely inhibited. In any case, these data suggest that expression of the Kdp system requires protein synthesis.

DISCUSSION

Until now, it has not been clear whether *S. faecalis* has multiple transport systems for K^+, although several types of mutants deficient in K^+ transport have been isolated (5). The principal reason for this is that genetic analysis of chromosomal genes is not feasible in *S. faecalis*. Detailed studies by Bakker and Harold (1) led to the conclusion that K^+ accumulation by *S. faecalis* requires both ATP and the proton motive force. In this paper I report that *S. faecalis* has a second transport system for K^+. The essential features of this system are as follows: (i) the system is driven by ATP (or a derivative of ATP) and does not require the proton motive force; and (ii) the system is normally repressed but can be derepressed by lowering the intracellular concentration of K^+. Based on a comparison of the characteristics of these two systems in *S. faecalis* with the systems of *E. coli*, I have designated them the Trk and Kdp systems.

The Kdp system was derepressed when the intracellular concentration of K^+ was lowered by monactin treatment. Therefore, one would expect the Kdp system to be expressed in cells grown on medium containing a very low concentration of K^+. In *S. faecalis*, the Trk system accumulates K^+ until the external K^+ concentration falls below 10 μM, and this system can establish a concentration gradient of K^+ as high as 50,000 (1). Therefore, the Kdp system should be found in cells grown on medium containing less than 10 μM K^+. Experiments to study this are in progress.

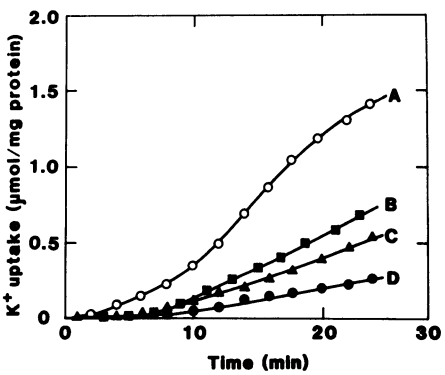

FIG. 8. Derepression of the Kdp system requires protein synthesis. Cells were treated with monactin in different media, as described in the text. Line A, no addition; line B, 5 μg of erythromycin per ml was added; line C, tryptone and yeast extract were omitted, 2 mM $MgSO_4$ was added, and the cells were then treated with DCCD and K^+ uptake was assayed as described in the legend to Fig. 6; line D, data from Fig. 6A. The protein concentrations in the reaction mixtures were as follows: line A, 0.22 mg/ml; line B, 0.19 mg/ml; line C, 0.27 mg/ml; line D, 0.20 mg/ml.

Recently, Epstein and Laimins (2) suggested that in *E. coli* the mutations of all *trk* mutants affect the TrkA system; this would mean that *E. coli* has only two transport systems for K^+, the TrkA and Kdp systems. My results show that *S. faecalis* also has two transport systems for K^+, which closely resemble those of *E. coli*. It is very interesting that *E. coli* and *S. faecalis* have analogous transport systems for K^+ despite the fact that these two bacteria are not closely related. The existence of two transport systems for K^+ has also been reported in *Paracoccus denitrificans* (3).

Finally, the physiological roles of K^+ transport systems should be mentioned. Recent studies in my laboratory have shown that the cytoplasmic pH in *S. faecalis* is regulated by both the H^+-ATPase and the K^+ transport system (9). As shown in Fig. 2, the activity of the Kdp system is very low at pH values below 7. This make it unlikely that the Kdp system regulates the cytoplasmic pH, because pH regulation must be active in acidic media in order to raise the cytoplasmic pH to 7.5. Although the evidence is not sufficient, it does suggest that the Trk system accumulates K^+ in order to regulate the cytoplasmic pH, whereas the Kdp system maintains the intracellular K^+ level when the Trk system is not sufficient for the task. Thus, it is possible that each system has its own function in bacterial K^+ transport systems. This may be the reason why bacteria have multiple transport systems for K^+.

ACKNOWLEDGMENTS

I am most grateful to Franklin M. Harold for valuable discussions and for help in the preparation of the manuscript. I also thank Tsutomu Unemoto for helpful discussions.

This work was supported by Grant-in-Aid for Special Research 411301 from The Ministry of Education, Science, and Culture (Japan).

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. **Epstein, W., and L. Laimins.** 1980. Potassium transport in *Escherichia coli*: diverse systems with common control by osmotic forces. Trends Biol. Sci. **5**:21–23.
3. **Erecinska, M., C. J. Deutsch, and J. S. Davis.** 1981. Energy coupling to K^+ transport in *Paracoccus denitrificans*. J. Biol. Chem. **256**:278–284.
4. **Harold, F. M.** 1977. Ion currents and physiological functions in microorganisms. Annu. Rev. Microbiol. **31**:181–203.
5. **Harold, F. M., and K. Altendorf.** 1974. Cation transport in bacteria: K^+, Na^+, and H^+. Curr. Top. Membr. Transp. **5**:1–50.
6. **Harold, F. M., and J. R. Baarda.** 1968. Effects of nigericin and monactin on cation permeability of *Streptococcus faecalis* and metabolic capacities of potassium-depleted cells. J. Bacteriol. **95**:816–823.
7. **Harold, F. M., and D. Papineau.** 1972. Cation transport and electrogenesis by *Streptococcus faecalis*. II. Proton and sodium extrusion. J. Membr. Biol. **8**:45–62.
8. **Kashket, E. R.** 1979. Active transport of thallous ions by *Streptococcus lactis*. J. Biol. Chem. **254**:8129–8131.
9. **Kobayashi, H., and T. Unemoto.** 1980. *Streptococcus faecalis* mutants defective in regulation of cytoplasmic pH. J. Bacteriol. **143**:1187–1193.
10. **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.
11. **Nakajima, H., I. Yamato, and Y. Anraku.** 1979. Quantitative analysis of potassium ion pool in *Escherichia coli* K-12. J. Biochem. (Tokyo) **85**:303–310.
12. **Rhoads, D. B., and W. Epstein.** 1977. Energy coupling to net K^+ transport in *Escherichia coli* K-12. J. Biol. Chem. **252**:1394–1401.
13. **Rhoads, D. B., A. Woo, and W. Epstein.** 1977. Discrimination between Rb^+ and K^+ by *Escherichia coli*. Biochim. Biophys. Acta **469**:45–51.
14. **Sorensen, E. N., and B. P. Rosen.** 1980. Effects of sodium and lithium ions on the potassium ion transport systems of *Escherichia coli*. Biochemistry **19**:1458–1462.