Bioenergetic Properties of Alkaline-tolerant and Alkalophilic Strains of *Bacillus firmus*

By ARTHUR A. GUFFANTI, ROBERT BLANCO, ROBERT A. BENENSON
AND TERRY A. KRULWICH*

Department of Biochemistry, Mount Sinai School of Medicine of the City University of New York, New York, New York 10029, U.S.A.

(Received 3 October 1979; revised 4 January 1980)

The protonmotive force, Na^+/H^+ antiport activity and α -aminoisobutyric acid transport were studied in alkaline-tolerant *Bacillus firmus* ATCC 14575 and in a newly isolated alkalophilic strain of the same species. The alkalophilic strain, which grew over a pH range from 8.0 to 10.5, maintained a cytoplasmic pH of 9.5 or below even at an external pH of 11.0. Evidence was obtained for an electrogenic Na^+/H^+ antiporter which could play a role in acidification of the cytoplasm as well as in generation of the transmembrane electrical potential. A non-alkalophilic strain, isolated after mutagenesis of the new alkalophile, lacked the Na^+/H^+ antiporter. The alkaline-tolerant strain of *B. firmus*, which grew at pH values between 6.5 and 8.5, did not maintain an acidified cytoplasm as the pH was raised above 8.0. A less active, apparently electroneutral, Na^+/H^+ exchange was found in this strain. Both the alkaline-tolerant and alkalophilic strains exhibited Na^+ -dependent transport of α -aminoisobutyric acid.

INTRODUCTION

The special bioenergetic problems posed by life at alkaline pH, especially the generation of the protonmotive force (p.m.f.) and its involvement in solute transport, have been studied in one alkaline-tolerant (Guffanti *et al.*, 1979*b*) and one alkalophilic species of *Bacillus* (Guffanti *et al.*, 1978, 1979*a*). The former, a *Bacillus circulans* strain, could grow at pH values between 6.5 and 9.5, while the latter, *Bacillus alcalophilus*, could only grow at alkaline pH values, from 8.0 to 11.5. The studies suggested that the upper limit of cytoplasmic pH that is compatible with viability is 9.0 to 9.5, which corresponds to the upper pH limit for growth of alkaline-tolerant organisms (Guffanti *et al.*, 1979*b*). The true alkalophile maintains a cytoplasmic pH of 9.0 to 9.5 as the pH is raised further, thus growing optimally under conditions in which the cytoplasm is more acidic than the external milieu (Guffanti *et al.*, 1978). In membrane vesicles this reversed ΔpH could only be generated in the presence of Na^+ ; work with vesicles and starved intact cells indicated that an electrogenic Na^+/H^+ antiporter is critically involved in the maintenance of an acidified cytoplasm (Mandel & Krulwich, 1979).

The p.m.f., the sum of the reversed ΔpH and the transmembrane electrical potential or $\Delta\psi$ (Mitchell, 1961), is very small in *B. alcalophilus*. The alkalophile must be able to energize transport, ATP synthesis, etc. using this small p.m.f. or some alternative mechanism. In previous studies, we found that α -aminoisobutyric acid (AIB) transport occurs in symport with Na^+ , energized by the $\Delta\psi$ (Guffanti *et al.*, 1978), and that β -galactosides are transported by an ATP-dependent mechanism (Guffanti *et al.*, 1979*a*). The mechanism of ATP synthesis in this species is currently under investigation in our laboratory.

Recently, we isolated a new alkalophilic strain of *Bacillus* in order to verify that the

bioenergetic patterns of *B. alcalophilus* are not some special property of that species. The new isolate was identified as an obligately alkalophilic strain of *Bacillus firmus*, a species that is typically alkaline-tolerant. Thus, it has been possible to compare directly alkaline-tolerant and truly alkalophilic strains of the same species. We have further been able to isolate a non-alkalophilic mutant of the new isolate. The findings of the previous studies have been extended to include evidence for the existence of an electrogenic Na^+/H^+ antiporter whose activity is correlated with the ability to grow at highly alkaline pH.

METHODS

Bacteria and growth conditions. *Bacillus firmus* ATCC 14575 was obtained from the American Type Culture Collection. A bacterium able to grow at pH 10.5 was isolated from garden soil by enrichment culture techniques, using alkaline medium containing L-malate as the carbon source, and was purified by single colony isolation from solid medium. On the basis of its morphological and physiological characteristics, the isolate has been identified by Dr Ruth Gordon (Waksman Institute of Microbiology, Rutgers University, New Brunswick, N.J., U.S.A.) as an alkalophilic strain of *B. firmus*. Several similar strains have recently been characterized by Gordon (1980). *Bacillus firmus* 14575 is a moderately alkaline-tolerant strain which grows at pH values between 6.5 and 8.5. The alkalophilic strain (*B. firmus* RAB) grows well between pH 8.0 and 10.5. The basal growth medium for *B. firmus* RAB was that described previously for *B. alcalophilus* (Guffanti *et al.*, 1978), adjusted to pH 10.5. *Bacillus firmus* 14575 was grown on a basal medium containing Tris and phosphate buffers (Guffanti *et al.*, 1979*b*) adjusted to pH 7.5. The carbon source for both organisms was 50 mM-L-malate, supplemented with 0.1 % (w/v) yeast extract, 0.2 % (w/v) peptone and 1 % (v/v) trace salts solution (Hegeman, 1966). Both organisms were grown at 30 °C with shaking at 200 rev. min⁻¹ in a New Brunswick G25 rotary shaker. Growth was followed turbidimetrically with a Klett-Summerson colorimeter (no. 42 filter).

A non-alkalophilic mutant of *B. firmus* RAB was isolated after mutagenesis with ethyl methanesulphonate, as described previously (Wolfson & Krulwich, 1972), and plating on L-malate-containing medium buffered to pH 5.5 with potassium phosphate. Organisms grown from a single colony on such plates were designated as *B. firmus* RABN; this mutant grew on L-malate-containing medium only over a range of external pH from 5.5 to 8.5.

Chemicals. Lysozyme (EC 3.2.1.17) (egg white), gramicidin, luciferin-firefly tails and *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) were from Sigma. [¹⁴C]Inulin (270 Ci g⁻¹, 10 TBq g⁻¹), ³H₂O (250 µCi ml⁻¹, 9.3 MBq ml⁻¹), [¹⁴C]methylamine hydrochloride (52.2 mCi mmol⁻¹, 1.93 GBq mmol⁻¹), 5,5-[2-¹⁴C]-dimethylloxazolidine-2,4-dione (DMO) (8.8 mCi mmol⁻¹, 326 MBq mmol⁻¹), ²²NaCl (10 mCi mmol⁻¹, 370 MBq mmol⁻¹), α-[1-¹⁴C]AIB (9.0 mCi mmol⁻¹, 333 MBq mmol⁻¹) and [³H]triphenylmethylphosphonium bromide (TPMP⁺) (18.3 mCi mmol⁻¹, 677 MBq mmol⁻¹) were from New England Nuclear. Non-radioactive TPMP⁺ was purchased from ICN-K&K Laboratories.

Assays. Transport of AIB was measured by filtration as described previously (Guffanti *et al.*, 1978), except that 500 µM-[¹⁴C]AIB (1 µCi µmol⁻¹, 37 kBq µmol⁻¹) was used. The transmembrane ΔpH was measured by the distribution of [¹⁴C]methylamine or [¹⁴C]DMO, in a flow dialysis assay modified according to Ramos *et al.* (1976). The transmembrane electrical potential was determined in a filtration assay (Schuldiner & Kaback, 1975) by measuring the accumulation of [³H]TPMP⁺. The total p.m.f. (Δμ_{H⁺}) was calculated from the equation Δμ_{H⁺} = Δψ - ZΔpH, where Z = 58.8 mV at 25 °C. Care was taken to include the controls described previously (Guffanti *et al.*, 1978) for measurements of Δψ, ΔpH and AIB uptake. The intracellular water volume, as determined by the method of Stock *et al.* (1977), was 10.2 and 10.8 µl cell H₂O (mg cell protein)⁻¹ for *B. firmus* strains 14575 and RAB, respectively. Cellular ATP was measured with the luciferin-luciferase assay (Stanley & Williams, 1969) in organisms extracted with perchloric acid (Cole *et al.*, 1967) as described elsewhere (Guffanti *et al.*, 1978). Protein was determined by the method of Lowry, using egg-white lysozyme as the standard.

The efflux of ²²Na⁺ was measured with starved organisms by a variation of the method of Schuldiner & Fishkes (1978). Organisms were washed and suspended in 25 mM-potassium carbonate buffer, pH 10.5, or 25 mM-potassium phosphate buffer, pH 8.0, at 1 mg protein ml⁻¹ and starved for 3 to 5 h by shaking at 200 rev. min⁻¹ at 30 °C. The starved organisms were loaded with 1 mM-²²NaCl at 4 °C for 1 h. Portions were removed and transferred to reaction vessels at 25 °C to which the appropriate additions were made. Samples (200 µl) were removed, filtered and washed rapidly with 25 mM-potassium carbonate buffer, pH 10.5 or potassium phosphate, pH 8.0. Filtration and washing were completed within 5 to 10 s. Slower rates of filtration led to considerable ²²Na⁺ leakage.

Tracings of the external pH were conducted as described by Niven & MacLeod (1978). Organisms were suspended at 5 to 10 mg protein ml⁻¹ and starved in a reaction mixture of 300 mM-choline chloride, 50 mM-

MgSO₄, 1 mM-Tris/HCl, and 20 µg carbonic anhydrase ml⁻¹. The suspension was kept anaerobic by bubbling with oxygen-free nitrogen, and the pH was adjusted with anaerobic solutions of HCl or choline hydroxide. The pH was measured with a Beckman Expandomatic pH meter (combination pH electrode 39012) connected to a strip chart recorder with full-scale deflection set at 0.2 pH unit. Additions of either 10 mM-NaCl or 10 mM-KCl were made from anaerobic solutions.

RESULTS

The Δ pH and $\Delta\psi$ were determined, and the cytoplasmic pH and p.m.f. ($\Delta\bar{\mu}_H+$) were calculated for each *B. firmus* strain over a range of external pH values. *Bacillus firmus* 14575 maintained a cytoplasmic pH of approximately 8.0 over an external pH range from 6.5 to 8.0 (Fig. 1). Thus in this pH range, a Δ pH, outside acid, was generated. At higher external pH values, the Δ pH was zero. The $\Delta\psi$ increased from -77 mV at pH 6.5 to -137 mV at pH 9.0 and then began to decline. By contrast, *B. firmus* RAB maintained a cytoplasmic pH of 9.5 or less over a range of external pH values from 8.0 to 11.0; the $\Delta\psi$ increased over that entire range from -89 to -145 mV (Fig. 2). The p.m.f. of the alkaline-tolerant strain was highest at neutral or slightly acidic external pH values; that of the alkalophile was highest at pH 9.5, the most alkaline pH at which a reversed Δ pH was not produced.

The possible existence of monovalent cation/H⁺ antiporters was then examined. At alkaline pH, such antiporters might be involved in the maintenance of the $\Delta\psi$ in *B. firmus* 14575 and of both the $\Delta\psi$ and reversed Δ pH in *B. firmus* RAB. First, suspensions of the organisms in lightly buffered medium at pH 8.0 or 10.5 were exposed to a sudden addition of NaCl or KCl to a final concentration of 10 mM. The conditions used were those described by Niven & MacLeod (1978). Suspensions of both strains at pH 8.0 and at pH 10.5 showed a slight acidification of the medium (approximately 0.01 pH units) upon addition of NaCl. No change was observed upon addition of KCl. Efflux of Na⁺ from starved organisms which had been loaded with ²²Na⁺ was then studied (Fig. 3). At pH 10.5 neither strain showed efflux of Na⁺ in the absence of energization. Upon addition of ascorbate/TMPD, ²²Na⁺ effluxed rapidly from *B. firmus* RAB (Fig. 3*a*). The efflux was markedly reduced by the lipophilic cation TPMP⁺, which was used to dissipate the $\Delta\psi$ without itself making the membrane permeable to Na⁺. *Bacillus firmus* 14575 exhibited a much slower, TPMP⁺-insensitive, efflux of ²²Na⁺ upon energization (Fig. 3*b*). Parallel measurements under the same conditions indicated that equivalent $\Delta\psi$ values, in the range -80 to -90 mV, were generated in the two strains upon addition of ascorbate/TMPD to the starved organisms. The relative ²²Na⁺ efflux exhibited by the two *B. firmus* strains was essentially the same at pH 8.0 as at pH 10.5 (Table 1). Moreover, use of valinomycin to cause dissipation of the $\Delta\psi$ resulted in levels of inhibition in *B. firmus* RAB similar to those observed with TPMP⁺. Valinomycin did not inhibit the more modest ²²Na⁺ efflux found in *B. firmus* 14575. However, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) [used at concentrations shown previously to cause transmembrane proton movement at alkaline pH (Guffanti *et al.*, 1978)] inhibited ²²Na⁺ efflux in this strain.

The non-alkalophilic mutant *B. firmus* RABN had been isolated because of the possibility that if a specific cation/proton antiporter facilitates growth at high pH by maintaining an acidified cytoplasm, then activity of that same antiporter might preclude growth of the alkalophile at neutral pH by causing acidification beyond some critical buffering capacity of the cytoplasm. A non-alkalophilic mutant might thus be expected to lack the relevant antiporter. The mutant exhibited no ²²Na⁺ efflux at either pH 8.0 or 10.5 under the conditions employed for the other two strains (Table 1). Moreover, addition of NaCl to suspensions of mutant RABN in lightly buffered medium failed to cause the slight acidification observed under the same conditions with *B. firmus* 14575 and *B. firmus* RAB (results not shown).

The final bioenergetic property studied in *B. firmus* 14575 and *B. firmus* RAB was an active transport system for AIB. Uptake of AIB occurred optimally at pH 7.5 in *B. firmus* 14575 and at pH 9.0 in *B. firmus* RAB. The AIB accumulated could be shown by extraction

Fig. 1

Fig. 2

Fig. 1. Effect of external pH on internal pH (●), $\Delta\psi$ (×), $Z\Delta pH$ (○) and $\Delta\bar{\mu}_{H^+}$ (▲) in *B. firmus* 14575. Organisms grown at pH 7.5 were washed and suspended in either 25 mM- $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ buffer (pH 9.0 to 11.0) or 25 mM-Tris/25 mM-sodium phosphate (pH 6.5 to 9.5). Measurements were made as described in Methods. All points are the average of at least two determinations.

Fig. 2. Effect of external pH on internal pH (●), $\Delta\psi$ (×), $Z\Delta pH$ (○) and $\Delta\bar{\mu}_{H^+}$ (▲) in *B. firmus* RAB. Measurements were made as described in Methods and in the legend to Fig. 1.

Fig. 3. Effect of an energy source and an inhibitor of $\Delta\psi$ on Na^+ extrusion in starved *B. firmus* RAB (a) and *B. firmus* 14575 (b). Organisms were starved at pH 10.5 as described in Methods and loaded with 1 mM- $^{23}\text{NaCl}$. Samples were taken, rapidly filtered and washed with 25 mM-potassium carbonate buffer, pH 10.5. No additions were made to control suspensions (●), while 20 mM-potassium ascorbate plus 2 mM-TMPD were added to other suspensions in the presence (■) or absence (○) of 1 mM-TPMP $^+$.

Table 1. *Effect of inhibitors on efflux of Na⁺*

Organisms were harvested and starved as described in Methods. After loading with 1 mM-²²NaCl for 1 h on ice at pH 8.0 or 10.5, the suspensions were divided into portions and warmed to 25 °C. Inhibitors (10 µM-valinomycin and 100 µM-CCCP) were added 10 min prior to the addition of the energy source (20 mM-potassium ascorbate plus 2 mM-TMPD). Samples (200 µl) were taken and rapidly filtered 15 s after the addition of the energy source. All values represent the average of 10 separate determinations.

Organism	Intracellular Na ⁺ concn [nmol (mg protein) ⁻¹]									
	pH 8.0					pH 10.5				
	Ascorbate/TMPD					Ascorbate/TMPD				
	No addition	No addition	No addition	+ Valinomycin	+ CCCP	No addition	No addition	+ Valinomycin	+ CCCP	
<i>B. firmus</i> 14575	11.0	8.2	11.2	8.6	10.5	9.3	9.3	9.3	12.6	
<i>B. firmus</i> RAB	10.2	1.8	9.9	4.9	—	1.1	1.1	4.5	—	
<i>B. firmus</i> RABN	10.5	11.9	10.4	12.2	—	10.0	10.0	11.6	—	

Table 2. *Effect of various ions on AIB uptake*

Organisms were grown on L-malate, washed and suspended in 25 mM-Tris/HCl, pH 9.0 (*B. firmus* RAB) or pH 7.5 (*B. firmus* 14575). The various salts were added to a final concentration of 10 mM.

Addition	AIB uptake [nmol min ⁻¹ (mg protein) ⁻¹]		Addition	AIB uptake [nmol min ⁻¹ (mg protein) ⁻¹]	
	<i>B. firmus</i> 14575	<i>B. firmus</i> RAB		<i>B. firmus</i> 14575	<i>B. firmus</i> RAB
None	6.3	11.8	CuCl ₂	2.8	8.9
NaCl	10.6	20.9	NiSO ₄	5.0	8.1
KCl	6.3	10.7	CaCl ₂	7.1	3.8
LiCl	5.6	9.5	NH ₄ Cl	6.2	7.7
MgCl ₂	6.6	10.6	FeCl ₂	3.1	7.4
MnCl ₂	5.8	6.5	FeCl ₃	6.2	4.5
CoCl ₂	6.7	6.2	RbCl	5.3	11.1

(Thompson, 1976) and chromatography (Pillay & Mehdi, 1970) to be identical to authentic AIB. Concentrative uptake of the solute was dependent on the presence of Na⁺ in both strains. This dependence was particularly clear when steady-state levels of accumulation were examined after 1 h of uptake. In the absence of Na⁺, *B. firmus* 14575 and *B. firmus* RAB accumulated AIB to 8.5 and 19.0 nmol h⁻¹ (mg protein)⁻¹, respectively. In the presence of Na⁺ (10 mM), *B. firmus* 14575 and *B. firmus* RAB accumulated AIB to 119.9 and 419.4 nmol h⁻¹ (mg protein)⁻¹, respectively. Other cations failed to stimulate the initial rate of AIB uptake (Table 2). In the presence of Na⁺, AIB uptake exhibited Michaelis-Menten kinetics with a *K_m* of 260 μM in *B. firmus* 14575 and 320 μM in *B. firmus* RAB. The AIB transport system was examined in greater detail in the alkalophilic strain. Gramicidin, an agent that dissipated the Δψ, completely inhibited AIB transport (results not shown). By contrast, a direct involvement of ATP in the AIB transport system was not found. *Bacillus firmus* RAB was incubated in medium containing 10 mM-sodium arsenate for 30 min at 30 °C. After washing, the treated and control organisms were assayed for cellular ATP content and AIB transport. Arsenate treatment lowered the ATP content from 3.35 to 0.33 nmol ATP (mg cell protein)⁻¹, while AIB transport was completely unaffected.

DISCUSSION

The major findings of previous studies on the p.m.f. generated by alkaline-tolerant and alkalophilic organisms have been confirmed. Again, growth of the two strains examined here occurred only at external pH values at which the cytoplasmic pH could be maintained at 9.5 or below. While both the alkaline-tolerant and alkalophilic strains exhibited substantial Δψ values above pH 9.5, only the alkalophilic strain maintained a reversed ΔpH.

We had proposed previously that one of the important differences between alkaline-tolerant and alkalophilic bacilli might be in the property of a cation/proton antiporter (Guffanti *et al.*, 1979*b*); subsequent investigations of an alkalophile indeed indicated a role for a Na⁺/H⁺ antiporter in acidification of the cytoplasm (or intravesicular space) of *B. alcalophilus* (Mandel & Krulwich, 1979). An electrogenic Na⁺/H⁺ antiporter could function to maintain an acidified cytoplasm as long as its activity returned more protons to the cytoplasm (or intravesicular space) than were being extruded by respiration. As a hypothetical example: suppose two protons were extruded during respiration and the antiporter, equally rapidly, exchanged three protons for two intracytoplasmic Na⁺ ions. The antiporter activity alone (2Na⁺ extruded/3H⁺ taken up) would be electrogenic in the direction that would allow its energization by the Δψ, and/or a gradient of Na⁺, where Na⁺_{in} > Na⁺_{out}, augmented by the transient gradient of protons produced by respiration. The total movement of ions that would result from respiration and the antiporter, in the example, would produce an acidified cytoplasm and a Δψ, outside positive.

Does a Na^+/H^+ antiporter function to acidify the cytoplasm in *B. firmus* RAB, and could the difference between the alkaline-tolerant and alkalophilic strains relate to differences in the antiporters? Indeed, the alkalophilic strain appears to possess a $\Delta\psi$ -dependent antiporter as indicated by: (i) Na^+ -induced proton efflux from cells and (ii) energy-dependent Na^+ efflux that was partially sensitive to TPMP⁺ or valinomycin (in the presence of K^+). That this antiporter activity is actually involved in maintenance of the acidified cytoplasm is strongly indicated by the observation that a non-alkalophilic mutant lacks the activity. By contrast, the naturally alkaline-tolerant strain *B. firmus* 14575 appears to possess a less active antiporter than *B. firmus* RAB. The Na^+/H^+ antiporter in *B. firmus* 14575 catalyses an energy-dependent, $\Delta\psi$ -independent Na^+ efflux, as indicated by its insensitivity to TPMP⁺ or valinomycin. This antiporter may be energized by the transient chemical gradient resulting from primary proton extrusion, since it was completely inhibited by CCCP. Thus, the Na^+/H^+ antiporter of *B. firmus* 14575 could not produce an acidified cytoplasm, in contrast to that of the alkalophile.

While these considerations all indicate that Na^+/H^+ antiporters with different properties correlate with the relative ability to grow at alkaline pH, a much more clear cut demonstration of Na^+ -dependent proton movements should be obtained in both strains. Such a demonstration will strengthen the inference that the inhibitor-sensitive Na^+ movements indicate Na^+/H^+ antiporter activity. In future studies, we intend to use membrane vesicles of known orientation, together with techniques that will facilitate monitoring of proton fluxes.

There are reports of electrogenic cation/proton antiporters in *Escherichia coli* (Schuldiner & Fishkes, 1978; Brey & Rosen, 1979) and *Halobacterium halobium* (Lanyi & MacDonald, 1976); similar antiporters described in other species of bacteria (Bhattacharyya & Barnes, 1978; Harold & Papineau, 1972; Niven & MacLeod, 1978) may also be electrogenic. A role for antiporters in the conversion of the ΔpH , produced by primary proton extrusion to a $\Delta\psi$, with $\Delta\text{pH}=0$, had been suggested previously in connection with studies of the p.m.f. in vesicles (Ramos & Kaback, 1977). A specific involvement of a Na^+/H^+ antiporter (Padan *et al.*, 1976; Schulachev, 1978; Schuldiner & Fishkes, 1978) and of the K^+/H^+ antiporter (Brey & Rosen, 1979) in the maintenance of cytoplasmic pH have been proposed in *E. coli*. The alkalophiles seem to offer an extreme biological situation in which the roles of the antiporters can be readily examined.

We are indebted to Dr Ruth Gordon for identifying the newly isolated alkalophile as a strain of *Bacillus firmus*. This work was supported in part by research grants PCM 7725586 and PCM 7810213 from the National Science Foundation. Dr Krulwich is the recipient of Research Career Development Award 5K04 GM00020 from the National Institutes of Health.

REFERENCES

- BHATTACHARYYA, P. & BARNES, E. M., JR (1978). Proton-coupled sodium uptake by membrane vesicles from *Azotobacter vinelandii*. *Journal of Biological Chemistry* **253**, 3848–3851.
- BREY, R. N. & ROSEN, B. P. (1979). Cation/proton antiport systems in *Escherichia coli*. *Journal of Biological Chemistry* **254**, 1957–1963.
- COLE, J., WIMPENNY, J. W. T. & HUGHES, D. E. (1967). The ATP pool in *Escherichia coli*. I. Measurement of the pool using a modified luciferase assay. *Biochimica et biophysica acta* **143**, 445–453.
- GORDON, R. E. (1980). 107 years of the genus *Bacillus*. In *The Aerobic Endospore-forming Bacteria: Classification and Identification* (in the Press). Edited by R. C. W. Berkeley & M. Goodfellow. London: Academic Press.
- GUFFANTI, A. A., SUSMAN, P., BLANCO, R. & KRULWICH, T. A. (1978). The protonmotive force and α -aminoisobutyric acid transport in an obligately alkalophilic bacterium. *Journal of Biological Chemistry* **253**, 708–715.
- GUFFANTI, A. A., BLANCO, R. & KRULWICH, T. A. (1979a). A requirement for ATP for β -galactoside transport by *Bacillus alcalophilus*. *Journal of Biological Chemistry* **254**, 1033–1037.
- GUFFANTI, A. A., MONTI, L., BLANCO, R., OZICK, D. & KRULWICH, T. A. (1979b). β -Galactoside transport in an alkaline-tolerant strain of *Bacillus circulans*. *Journal of General Microbiology* **112**, 161–169.

- HAROLD, F. M. & PAPINEAU, D. (1972). Cation transport and electrogenesis by *Streptococcus faecalis*. II. Proton and sodium extrusion. *Journal of Membrane Biology* **8**, 45–62.
- HEGEMAN, G. D. (1966). Synthesis of the enzymes of the mandelate pathway by *Pseudomonas putida*. I. Synthesis of enzymes by the wild type. *Journal of Bacteriology* **91**, 1140–1154.
- LANYI, J. K. & MACDONALD, R. E. (1976). Existence of electrogenic hydrogen ion/sodium ion antiport in *Halobacterium halobium* cell envelope vesicles. *Biochemistry* **15**, 4608–4614.
- MANDEL, K. G. & KRULWICH, T. A. (1979). Monovalent cation-proton antiports in membrane vesicles of *Bacillus alcalophilus*. *Abstracts of XIth International Congress of Biochemistry, Toronto, Canada*, p. 449.
- MITCHELL, P. (1961). Coupling of phosphorylation to electron and hydrogen transfer by a chemiosmotic type of mechanism. *Nature, London* **191**, 141–148.
- NIVEN, D. F. & MACLEOD, R. A. (1978). Sodium ion-proton antiport in a marine bacterium. *Journal of Bacteriology* **134**, 737–743.
- PADAN, E., ZILBERSTEIN, D. & ROTTENBERG, H. (1976). The proton electrochemical gradient in *Escherichia coli* cells. *European Journal of Biochemistry* **63**, 533–541.
- PILLAY, D. T. N. & MEHDI, R. (1970). Separation of amino acids by thin-layer chromatography. *Journal of Chromatography* **47**, 119–123.
- RAMOS, S. & KABACK, H. R. (1977). The electrochemical proton gradient in *Escherichia coli* membrane vesicles. *Biochemistry* **16**, 848–854.
- RAMOS, S., SCHULDINER, S. & KABACK, H. R. (1976). The electrical gradient of protons and its relationship to active transport in *Escherichia coli* membrane vesicles. *Proceedings of the National Academy of Sciences of the United States of America* **73**, 1892–1896.
- SCHULACHEV, V. P. (1978). Membrane linked energy buffering as the biological function of Na^+/K^+ gradient. *FEBS Letters* **87**, 171–179.
- SCHULDINER, S. & FISHKES, H. (1978). Sodium-proton antiport in isolated membrane vesicles of *Escherichia coli*. *Biochemistry* **17**, 706–711.
- SCHULDINER, S. & KABACK, H. R. (1975). Membrane potential and active transport in membrane vesicles from *Escherichia coli*. *Biochemistry* **14**, 5451–5461.
- STANLEY, P. E. & WILLIAMS, S. G. (1969). Use of the liquid scintillation spectrometer for determining adenosine triphosphate by the luciferase enzyme. *Analytical Biochemistry* **29**, 381–392.
- STOCK, J. B., RAUCH, B. & ROSEMAN, S. (1977). Periplasmic space in *Salmonella typhimurium* and *Escherichia coli*. *Journal of Biological Chemistry* **252**, 7850–7861.
- THOMPSON, J. (1976). Characteristics and energy requirements of an α -aminoisobutyric acid transport system in *Streptococcus lactis*. *Journal of Bacteriology* **127**, 719–730.
- WOLFSON, P. J. & KRULWICH, T. A. (1972). Inhibition of isocitrate lyase: the basis for inhibition of growth of two *Arthrobacter* species by pyruvate. *Journal of Bacteriology* **112**, 356–364.