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Buffering capacity and membrane H^+ conductance of acetic acid bacteria

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SUMMARY

Buffering capacities and membrane conductance to H^+ were measured in *Acetobacter aceti* ATCC 15973 and *Gluconobacter oxydans* ATCC 621 by a pulse technique. In both strains the buffering capacity of intact cells was a significant proportion of the total buffering capacity, but the magnitude of the buffering capacity varied between one species and another. Over the pH range studied, 4.02 to 8.15, *Gluconobacter oxydans*, which oxidizes sugars and alcohols to acids and accumulates them, showed lower values of buffering capacities and membrane conductance to protons than *Acetobacter aceti*, which oxidizes these substrates completely to CO_2 and H_2O.

INTRODUCION

Bacterial adaptability has enabled industries to optimize environmental conditions in order to increase specific product formation. Most industrial fermentations have been conducted under acid conditions in order to avoid contamination and/or to improve industrial production [2,3,10,12]. On the other hand, basic scientific researchers have been interested in the mechanisms that allow bacteria to survive and work under extreme conditions. One of these conditions is pH. Buffering capacity measurements may help us to understand how bacteria survive at different pH values.

Zychlinsky and Matin [13] suggested that the cytoplasmic buffering power of *Thiobacillus acidophilus* might be part of the pH homeostatic mechanism of this acidophile. Krulwich et al. [4] observed significantly higher Bi (internal buffering capacity) values in alcalophilic bacteria than in neutrophilic species at high external pH values. Maloney [6] measured buffering capacity and membrane proton conductance in the anaerobic bacterium *Streptococcus (Lactococcus) lactis* over the pH range 3.7–8.5. In a previous study, we showed that buffering capacity and membrane conductance to H^+ vary markedly from one strain to another [9]. These results focussed our attention on the available data on the buffering power and passive proton conductance of bacterial cells over a wide range of external pH values.

We were interested in studying these parameters for acetic acid bacteria due to their industrial significance and the fact that these bacteria are acid tolerant, they grow at pH as low as 4, with an optimum between pH 5 and 6. They can also grow at pH 7. They are Gram-negative or Gram-variable aerobic bacteria which oxidize ethanol to acetic acid in neutral or acid media. Many carbohydrates and primary and secondary alcohols can also serve as energy sources, their oxidation characteristically resulting in the transient or permanent accumulation of partly oxidized organic products. Certain oxidative products of acetic acid bacteria are industrially important, for example: gluconic acid, which is used by the pharmaceutical industry; sorbose, which is used as a suspending agent for certain pharmaceuticals, and is an intermediate in the manufacture of L-ascorbic acid (vitamin C). However their major industrial use is in the manufacture of vinegar, by the acetification of ethanol-containing materials (e.g. wine, cider) [1,2,12].

The experiments reported here provide quantitative estimates of buffering capacity and membrane conductance to H^+ of two strains of acetic acid bacteria over a wide range of pH (4.02–8.15). We used the method in which the decay of an acid pulse is used for determination of both parameters [6,9].

MATERIALS AND METHODS

Bacterial strains and growth conditions

Acetobacter aceti subsp. *aceti* ATCC 15973 and *Gluconobacter oxydans* subsp. *suboxydans* ATCC 621 were used in these experiments. Cells were grown aerobically, with vigorous shaking, to early stationary phase in a medium containing (per liter): yeast extract 5 g; mannitol 25 g and peptone of meat 3 g. They were kept on the solid medium at 4 °C and transferred monthly [12].

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Chemicals

Valinomycin and carbonic anhydrase were from Sigma Chemical Co., St Louis, MO, USA. All other chemicals were obtained from commercial sources. Valinomycin was used at a final concentration of 10 μM and added to cell suspensions as small volumes of concentrated stocks in acetone; final acetone concentrations did not exceed 0.2%. Carbonic anhydrase was prepared at 20 mg ml^{-1} in 300 mM KCl.

Preparation of non-proliferating cell suspensions (NPC)

Cells were harvested in the early stationary phase of growth and washed three times using 300 mM KCl. The washed cells were treated with 3 mM EDTA and resuspended in 300 mM KCl [9].

Measurement of cell protein

Protein content was determined according to Lowry et al. [5].

Measurement of buffering capacity and membrane H^+ conductance

Buffering capacity and membrane conductance to H^+ of these bacteria were measured by an acid-pulse technique, as described and discussed elsewhere [6–9,11]. Buffering power and membrane conductance to H^+ are presented as functions of external pH. The smooth curves that described behaviour of these parameters were obtained from a polynomic regression.

RESULTS AND DISCUSSION

Buffering capacity and membrane conductance to protons of non-proliferating cells of *Acetobacter aceti* ATCC 15973 were measured at pH values of 4.10–8.15. The pH range studied for titrations of *Gluconobacter oxydans* ATCC 621 cells was 4.02–7.93.

Data presented here and elsewhere [4,6,11,13] show that the internal and cell-surface buffering capacities of bacteria vary markedly between one species and another. Our results also confirm the common belief that the external buffering capacity (Bo) was a significant proportion of the total buffering capacity (Bt). Figure 1 summarizes measurements of Bo and Bt as a function of pH for each strain studied. The individual estimates of the internal buffering capacity (Bi) are not shown; instead, they were calculated as the difference between the two smooth curves that described the behaviour of Bo and Bt. As Bi almost certainly reflects the presence of proteins, nucleic acids and other molecules with phosphate, carboxyl and amino groups, it is important to obtain reproducible values of this magnitude to carry out Bi determinations under strictly controlled growth conditions.

A. aceti exhibited a maximum value of Bi of 290 nmol H^+ per pH unit per mg of protein at pH 4.70 and a minimum value of 12 nmol H^+ per pH unit per mg of protein at pH 8.15. Over this pH range there was a considerable change in Bi with a marked tendency to increase as pH became more acidic. The maximum Bi value was 24-fold greater than the minimum value. A similar tendency has been noted by others, who have measured Bi over this same range for *S. lactis* [6], *S. marcescens* [9] and in the pH range from 4 to 6 for *E. coli*, *B. acidocaldarius* and *B. stearothermophilus* [4]. As noted by Maloney [6], Bi of some bacteria can be calculated accurately by an empirical formula, over a specific pH range. We report the following empirical formula: $Bi = (-51.5)\ pH + 407$, to calculate Bi of *G. oxydans* as a function of external pH, over the pH range from 4 to 7. Thus, as pH fell from 7 to 4, Bi rose 4.3-fold, from 46.5 to 201 nmol H^+ per pH unit per mg of protein. These acetic bacteria exhibited lower Bi values than those reported for *E. coli* [4] and for *S. marcescens* [9] over the same pH range.

Passive proton conductance of these acetic bacteria, like *S. marcescens* [9], was sensitive to proton concentration at the external surface as well as buffering capacity over the pH range studied (Fig. 2). The value of membrane conductance determines the rate at which H^+ leaks inward, and the balance between Cm, the value of the proton-motive gradient, and the rate of outward pumping will determine whether a bacterial cell can sustain an appropriate pH gradient under acid or alkaline conditions. *A. aceti* membrane conductance values were 0.45–1.88 nmol H^+/s per pH unit per mg protein over the pH range studied (data from smooth curve that described behaviour of C_m^H). These values were comparable to those found for *S. marcescens* GP [9], over the same pH range. *G. oxydans* presented a different smooth curve that described behaviour of C_m^H. H^+ conductance increased as pH became more acidic, with a maximum of 1.56 nmol H^+/s per pH unit per mg protein at pH 4.02 and a minimum of 0.40 nmol H^+/s per pH unit per mg protein at pH 7.58. Between pH from 7 to 4 we can calculate membrane conductance to protons for *G. oxydans* by the empirical formula $C_m^H = (-0.38)\ pH + 3.06$. Thus, as pH fell from 7 to 4, passive membrane conductance to protons rose 3.8-fold, from 0.4 to 1.54 nmol H^+/s per pH unit per mg protein. Passive proton conductance of *G. oxydans* was 75% that of *A. aceti*. over the acidic range of pH.

These results show that buffering capacities and membrane conductance to protons of *G. oxydans* ATCC 621 were lower than in *A. aceti* ATCC 15973. *Acetobacter* and *Gluconobacter* are known for their direct oxidative capacity on sugars, alcohols and steroids [1]. These bacteria share the abilities to form acids by incomplete oxidation of sugars or alcohols and to excrete these acids, either transiently or into the medium as non-utilizable end products. They are distinguishable by their acid tolerance [12]. *Acetobacter* and *Gluconobacter* produce alcohol dehydrogenase, glucose dehydrogenase and other polyol dehydrogenases, which contain a prosthetic group, methoxatine or pyrrolquinoline quinone (PQQ). The enzymes are located on the outer membrane and they catalyze the oxidation of ethanol, glycerol, or glucose to the respective acids. In these reactions, the electrons are supplied by the electron transport chain, and the protons are extruded into the periplasmic space. Methoxatine can also reach the nutrient medium and can be found in vinegar. The production of acetic acid from ethanol is high with *Acetobacter* strains whereas the oxidative and ketogenic activities are high in *Gluconobacter* and weak in *Acetobacter*. Only *Acetobacter* oxidizes acetic acid and lactic acid to CO_2 and H_2O. Over the pH range 4.5–5, in which maximum production of acid takes place, Bo of

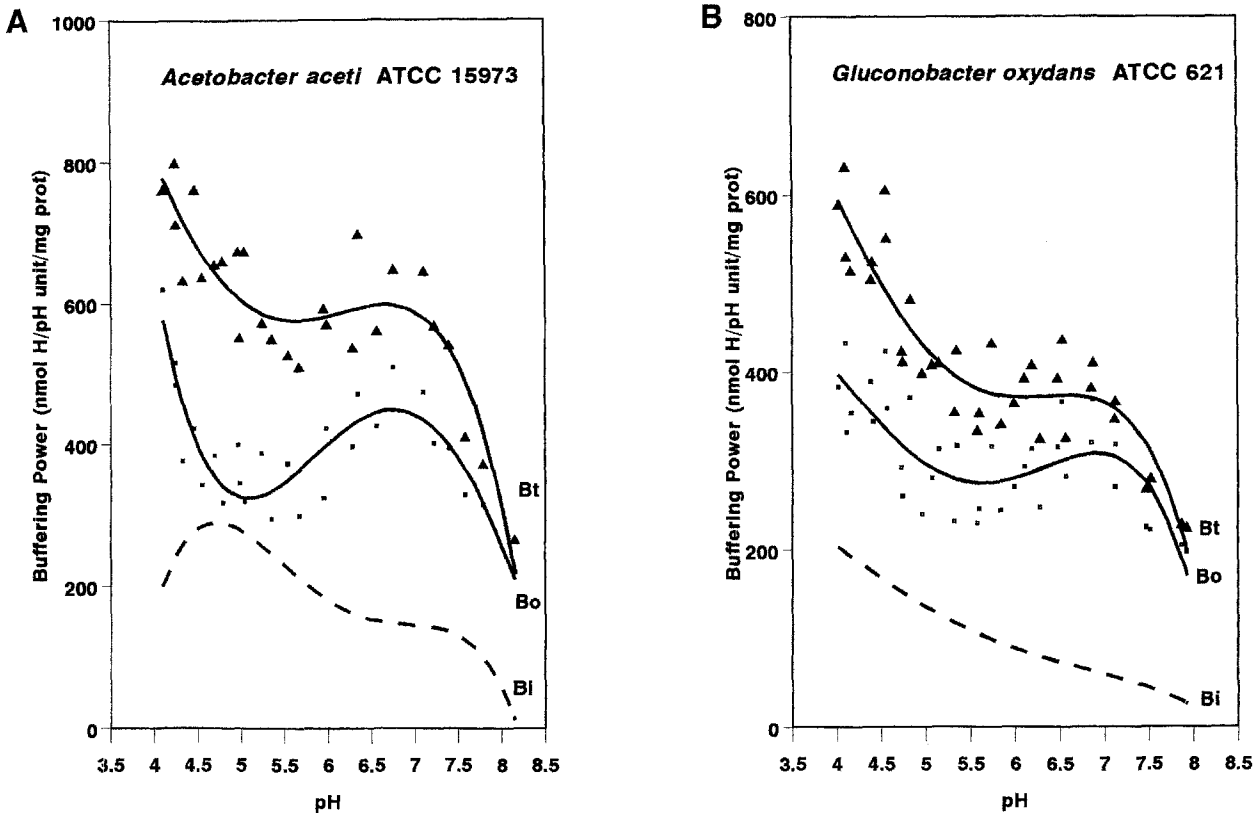

Fig. 1. Bo, Bt and Bi values for (A) *A. aceti* ATCC 15973 and (B) *G. oxydans* ATCC 621 over a range of external pH values. Bo and Bt values were determined as described in the text. Symbols represent data from independent experiments.

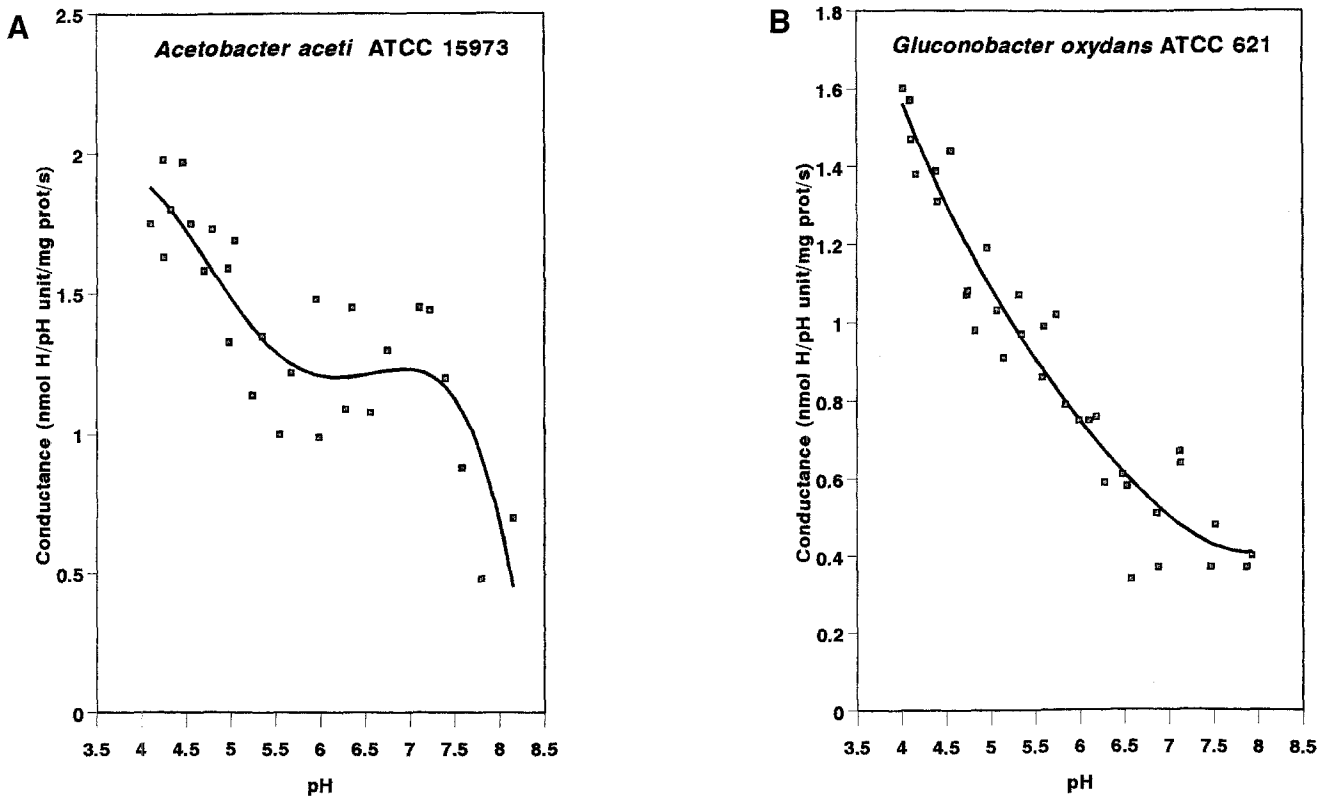

Fig. 2. Membrane conductance to H^+ of (A) *A. aceti* ATCC 15973 and (B) *G. oxydans* ATCC 621.

both bacteria were comparable (data from smooth curves) but *Gluconobacter* presented lower Bi values than *Acetobacter*. Although the physiological meaning of Bi is not known, our results suggest that buffering power and membrane conductance to protons of bacteria that accumulate acids because they lack the oxidative capacity to convert acetic and lactic acids to CO_2 and H_2O, are lower than in bacteria with complete oxidative metabolism.

REFERENCES

1 De Ley, J. and K. Kersters. 1964. Oxidation of aliphatic glycols by acetic acid bacteria. Bacteriol. Rev. 28: 164–180.
2 Demain, A.L. and N.A. Solomon (eds). 1985. Biology of Industrial Microorganisms, pp. 143–188, The Benjamin/Cummings Publishing Co., Menlo Park, CA.
3 Hawkes, L.E. and A.H. Linton (eds). 1971. The industrial uses of micro-organisms. In: Micro-organisms: Function, Form and Environment, pp. 680–697, Edward Arnold (Publishers), London.
4 Krulwich, T.A., R. Agus, M. Schneier and A.A. Guffanti. 1985. Buffering capacity of bacilli that grow at different pH ranges. J. Bacteriol. 162: 768–772.
5 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.
6 Maloney, P. 1979. Membrane H^+ conductance of *Streptococcus lactis*. J. Bacteriol. 140: 197–205.
7 Mitchell, P. and J. Moyle. 1967a. Acid-base titration across the membrane system of rat-liver mitochondria: catalysis by uncouplers. Biochem. J. 104: 588–600.
8 Mitchell, P. and J. Moyle. 1967b. Respiration-driven proton translocation in rat liver mitochondria. Biochem. J. 105: 1147–1162.
9 Rius, N., M. Solé, A. Francia and J.G. Lorén. 1994. Buffering capacity of pigmented and non-pigmented strains of *Serratia marcescens*. Appl. Environ. Microbiol. 60: 2152–2154.
10 Russell, J.B. 1992. Another explanation for the toxicity of fermentation acids at low pH: anion accumulation versus uncoupling. J. Appl. Bacteriol. 73: 363–370.
11 Scholes, P. and P. Mitchell. 1970. Acid-base titration across the membrane of *Micrococcus denitrificans*: factors affecting the effective proton conductance and the respiratory rate. J. Bioenerg. 1: 61–72.
12 Swings, J. 1992. The genera *Acetobacter* and *Gluconobacter*. In: The Prokaryotes (Balows, A., H.G. Trüper, M. Dworkin, W. Harder and K.H. Schleifer, eds), pp. 2268–2286, Springer-Verlag, New York.
13 Zychlinsky, E. and A. Matin. 1983. Cytoplasmic pH homeostasis in an acidophilic bacterium, *Thiobacillus acidophilus*. J. Bacteriol. 156: 1352–1355.