Journal of Industrial Microbiology (1995) 15, 411–413

Buffering capacity and membrane H^+ conductance of *Zymomonas mobilis*

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Buffering power and membrane conductance to H^+ were measured in *Zymomonas mobilis* subsp *mobilis* ATCC 29191 by a pulse technique. Over the pH range studied, from 4.02 to 7.44, *Z. mobilis* presented very high values of cytoplasmic buffering capacity; it was a significant proportion of the total buffering capacity. These results support the idea that the cytoplasmic buffering power might be part of the pH homeostatic mechanism.

Keywords: buffering capacity; H^+ conductance; *Zymomonas mobilis*

Introduction

In previous papers, we reported on our studies on buffering capacity and membrane conductance to protons of acetic acid and lactic acid bacteria. For acetic acid bacteria we showed that these parameters vary depending on metabolic activities. *Gluconobacter oxydans*, which oxidises sugars and alcohols to acids and accumulates them, presented lower values of buffering capacity and membrane conductance to protons that *Acetobacter aceti*, which oxidises such compounds completely to CO_2 and H_2O [10]. On the other hand, buffering capacity and membrane conductance to H^+ for the acidophilic *Lactobacillus acidophilus* was higher than for neutrophilic *Enterococcus faecalis* [9] and *Streptococcus lactis* [6]. All of these bacteria are homofermentative, yielding mainly lactic acid from glucose. In view of our interest in bacteria that grow and/or tolerate acidic conditions and the suggestion by Zychlinsky and Matin [17] that the cytoplasmic buffering power of *Thiobacillus acidophilus* might be part of the pH homeostatic mechanism of this acidophile, we report here measurements of buffering capacity and membrane conductance to protons for *Zymomonas mobilis*.

Z. mobilis is a neutrophilic Gram-negative bacterium with unusual physiological and biochemical properties, as reviewed by Swings and DeLey [16]. The wide pH range for growth, from 3.5 to 7.5, and the acid tolerance are both quite typical for this bacterium. The original pH in the liquid standard medium is 6.4. The final pH after 24 h at 27° C is 4.8. Genetically, phenotypically, and ecologically, *Zymomonas* is related to acetic acid bacteria *Gluconobacter* and *Acetobacter*. They all occur in acidic, sugary, and alcohol-containing niches such as tropical plant juices and beer. *Zymomonas* more closely resembles *Gluconobacter* than *Acetobacter* because of its polar flagella, its incomplete tricarboxylic acid cycle, and the occurrence of the Entner–Doudoroff pathway. It has been suggested that *Zymomonas* and the acetic acid bacteria might be derived from a common aerobic ancestor [12,16]. This has been corroborated by rRNA hybridization studies [3]. Following the Entner–Doudoroff pathway, *Z. mobilis* catabolizes a high percentage (95–98%) of the substrate carbon to ethanol and CO_2 and only a small percentage (2–5%) is incorporated into cell mass. A further exceptional property of *Z. mobilis* is its tolerance toward high concentrations of its substrate and product [2,16]. This bacterium performs a highly productive ethanol fermentation and its industrial applications can be extended to other substrates and products.

The quantitative estimates of buffering capacity reported here showed that *Z. mobilis* exhibited comparable values of cytoplasmic buffering power to the acidophilic bacterium *Lactobacillus acidophilus* [9] and a decrease in cytoplasmic buffering capacity as the external pH increased from pH 4 to 7.5, as noted by others for acidophilic and for acid-tolerant bacteria [6,10]. We have used the method in which the decay of an acid pulse is used for determination of buffering capacity and membrance conductance to protons [6, 8].

Materials and methods

Bacterial strain and growth conditions

Zymomonas mobilis subsp *mobilis* ATCC 29191 was used in these experiments. Cells were grown to early stationary phase in Standard Medium [16] and then washed three times in 300 mM KCl.

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 in 300 mM KCl. The washed cells

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Received 24 March 1995; accepted 3 June 1995

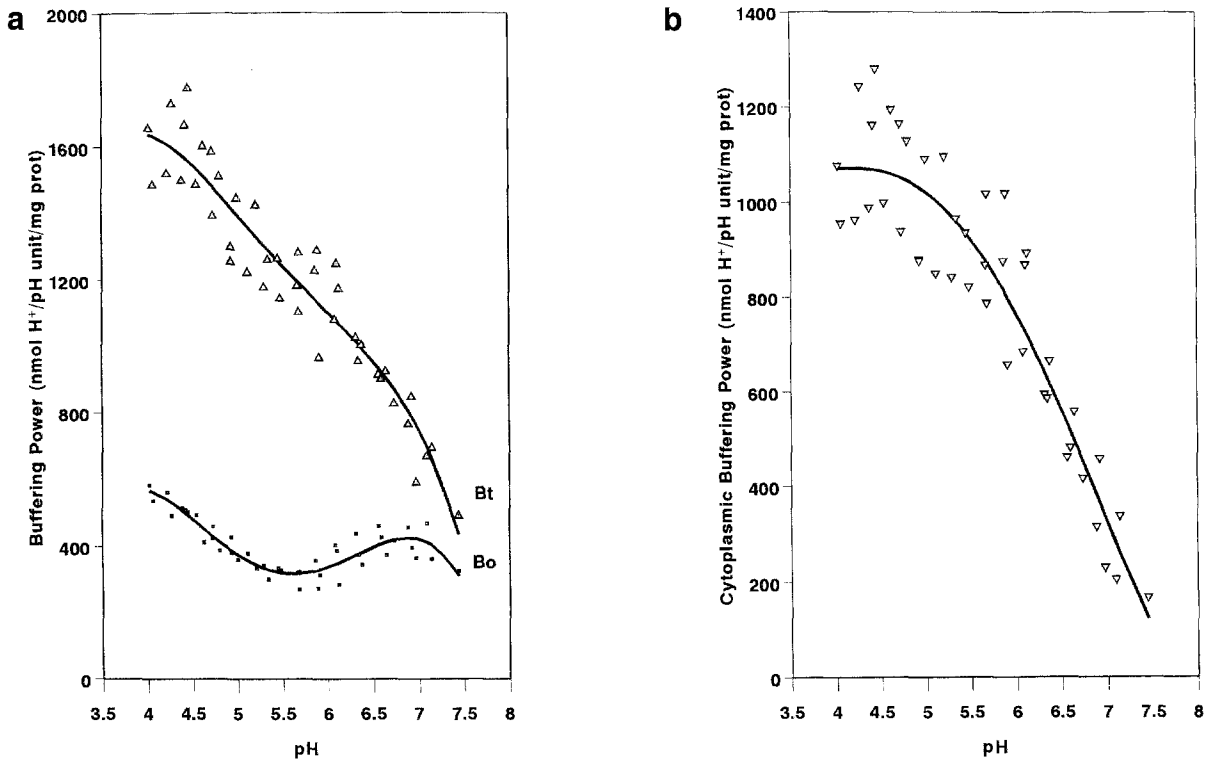

Figure 1 Buffering capacity for *Zymomonas mobilis*. (a) Bo and Bt, and (b) Bi values over the pH range 4.0–7.4. Bo and Bt values were determined as described in the text. Symbols represent data from independent experiments. Bi was calculated as the difference between the values of Bt and Bo. Cell protein was 5.2 mg ml^{-1}

were treated with 3 mM EDTA and suspended in 300 mM KCl [8].

Measurement of cell protein

Protein content was determined according to the method described by Lowry *et al* [5].

Measurement of buffering capacity and membrane conductance to H^+

Buffering capacity and membrane conductance to H^+ of *Z. mobilis* were measured by an acid-pulse technique as previously described [6–8, 15]. Buffering capacity and membrane conductance to protons are presented as functions of external pH. The smooth curves that describe the behaviour of these parameters were obtained from a polynomic regression.

Results and discussion

The major goal of these experiments was to provide a quantitative description of cytoplasmic buffering capacity over a wide range of pH (4.02–7.44). The results (Figure 1) indicate that for *Z. mobilis* internal buffering capacity (Bi) was a significant proportion of the total buffering capacity (Bt) and that Bi decreased with increasing pH in the range studied. On the other hand, external buffering power (Bo) varied slightly over this pH range in comparison to Bt and Bi, but Bo values were not quite different from Bo values found for acetic acid bacteria [10]. The pH of the standard medium fell from 6.4 to 4.8 for the first 24 h of growth of *Z. mobilis* and the final pH after 3 days at 27° C was 4.8–5.2. Thus, these bacteria may possess protection mechanisms against changes in external pH. One of these mechanisms could be the increase in cytoplasmic buffering power when pH_{out} became more acidic [17].

The values of cytoplasmic buffering capacity (Bi) reported here for *Z. mobilis* were comparable to the values found by Krulwich *et al* [4] for a well buffered bacterium, *Bacillus subtilis*, and by us [9] for the acidophilic bacterium *Lactobacillus acidophilus*. Clearly, the Bi of *Z. mobilis* was far greater than those of acetic acid bacteria [10].

Figure 2 shows that passive proton conductance of *Z. mobilis*, just like *Serratia marcescens* [8], *A. aceti* and *G. oxydans* [10], was sensitive to proton concentration at the external surface, over the pH range studied. *Z. mobilis* presented higher membrane H^+ conductance (C^H_m) values than those of acetic acid bacteria [10].

Zymomonas and acetic acid bacteria complement each other, since *Zymomonas* produces ethanol, which the acetic acid bacteria in turn, oxidize. The presence of pyruvate decarboxylase and alcohol dehydrogenase enables *Zymomonas mobilis* to perform a pure ethanol fermentation. The organism can produce high concentrations of ethanol, up to 13% (w/v) [11]. Thus, *Z. mobilis* possesses protection mechanisms against this toxic fermentative end product. Large amounts of hopanoids, pentacyclic triterpenoids, occur in *Z. mobilis* [1,13,14]. Probably these substances stabilize the cell membrane against the dissolving effect of ethanol. In this paper, we describe a further exceptional property of *Z. mobilis*, its high cytoplasmic buffering power

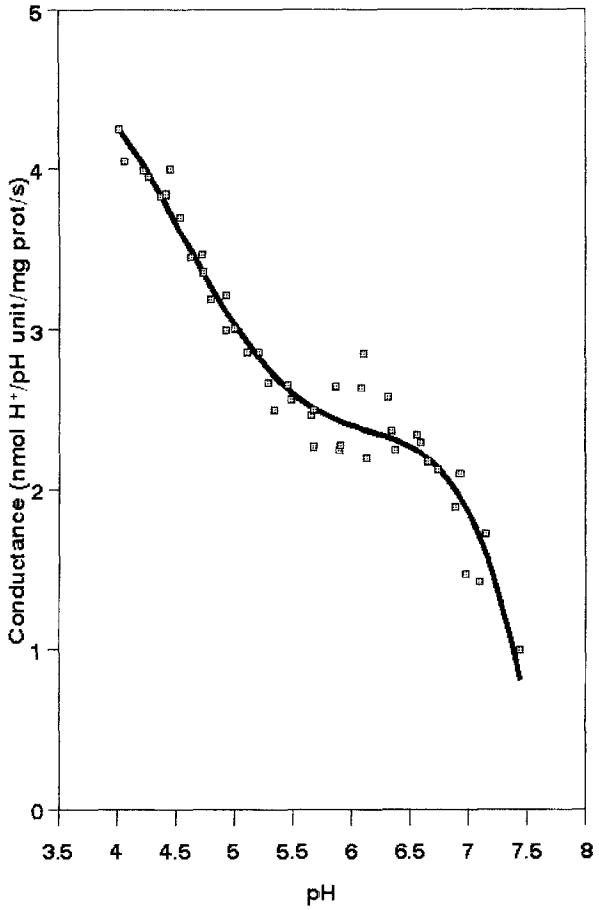

Figure 2 Membrane conductance to H^+ for *Z. mobilis*

that might protect the cells against external acidic conditions.

Acknowledgements

We thank Montserrat Solé and Alicia Francia for advice and encouragement. We are grateful to Robin Rycroft for expert criticism during the preparation of this manuscript.

References

1 Bringer S, T Härtner, K Poralla and H Sahm. 1985. Influence of ethanol on the hopanoid content and the fatty acid pattern in batch and continuous cultures of *Zymomonas mobilis*. Arch Microbiol 140: 312–316.
2 Bringer-Meyer SH and H Sahm. 1988. Metabolic shifts in *Zymomonas mobilis* in response to growth conditions. FEMS Microbiol Rev 54: 131–142.
3 Gillis M and J De Ley. 1980. Intra- and intergenic similarities of the ribosomal ribonucleic acid cistrons of *Acetobacter* and *Gluconobacter*. Int J Syst Bacteriol 30: 7–27.
4 Krulwich TA, R Agus, M Schneier and AA Guffanti. 1985. Buffering capacity of bacilli that grow at different pH ranges. J Bacteriol 162: 768–772.
5 Lowry OH, NJ Rosebrough, AL Farr and RJ 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. 1967. Acid-base titration across the membrane system of rat-liver mitochondria: catalysis by uncouplers. Biochem J 104: 588–600.
8 Rius N, M Solé, A Francia and JG Lorén. 1994. Buffering capacity of pigmented and non-pigmented strains of *Serratia marcescens*. Appl Environ Microbiol 60: 2152–2154.
9 Rius N, M Solé, A Francia and JG Lorén. 1994. Buffering capacity of lactic acid bacteria. FEMS Microbiol Lett 120: 291–296.
10 Rius N, A Francia, M Solé and JG Lorén. 1995. Buffering capacity and membrane H^+ conductance of acetic acid bacteria. J Ind Microbiol 14: 17–20.
11 Rogers PL, KJ Lee, ML Skotnicki and DE Tribe. 1982. Ethanol production by *Zymomonas mobilis*. Adv Biochem Eng 23: 37–84.
12 Sahm H, S Bringer-Meyer and G Sprenger. 1992. The genus *Zymomonas*. In: The Prokaryotes (Balows A, HG Trüper, M Dworkin, W Harder and KH Schleifer, eds), pp 2287–2301, Springer-Verlag, New York.
13 Schmidt A, S Bringer-Meyer, K Poralla and H Sahm. 1986. Effects of alcohols and temperature on the hopanoid content of *Zymomonas mobilis*. Appl Microbiol Biotechnol 25: 32–36.
14 Schmidt A, S Bringer-Meyer, K Poralla and H Sahm. 1989. Influence of ethanol on the activities of 3-hydroxy-3-methylglutaryl-coenzyme A-reductase and squalene-hopene-cyclase in *Zymomonas mobilis*. Appl Microbiol Biotechnol 30: 170–175.
15 Scholes P and P Mitchell. 1970. Acid-base tritation across the membrane of *Micrococcus denitrificans:* factors affecting the effective proton conductance and the respiratory rate. J Bioenerg 1: 61–72.
16 Swings J and J De Ley. 1977. The biology of *Zymomonas*. Bacteriol Rev 41: 1–46.
17 Zychlinsky E and A Matin. 1983. Cytoplasmic pH homeostasis in an acidophilic bacterium, *Thiobacillus acidophilus*. J Bacteriol. 156: 1352–1355.