Metabolic and energetic aspects of the growth response of *Streptococcus rattus* to environmental acidification in anaerobic continuous culture

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*Streptococcus rattus,* a serotype b strain of mutans streptococci, was grown in an anaerobic glucose-limited chemostat. The molar growth yield of glucose [\(\text{Y}_{\text{glucose}}\) g dry wt (mol glucose)] together with the maximum growth yields [\(\text{Y}_{\max}\)] and maintenance coefficients for glucose utilization and calculated ATP generation were estimated as a function of pH. When the pH was lowered from 7.0 to 5.0, \(\text{Y}_{\text{glucose}}\) decreased, with a concomitant gradual change in the composition of the end product from a mixture of formate, acetate and ethanol to one mostly of lactate. Whereas the \(\text{Y}_{\max}\) for glucose decreased without any change in the \(\text{Y}_{\max}\) for ATP on acidification, both of the maintenance coefficients markedly increased. Kinetic and immunochemical examinations indicated the presence of an \(\text{F}_{1}\text{F}_{0}\)-type proton-translocating ATPase in the membrane fraction prepared from bacterial cells grown under acidic conditions; no detectable level of the enzyme was found in cells grown at neutral pH. However, when incubated with glucose under non-growing conditions, these acid-adapted and unadapted cells showed an insignificant difference in the ability to maintain the intracellular pH alkaline relative to the acidic environments. These results suggest that the organism responds and adapts to environmental acidification by sacrificing some energy cost in terms of both the efficiency of glucose utilization to generate ATP and the extra maintenance required to continue biomass production as efficiently as under neutral pH.

**Keywords:** *Streptococcus rattus,* acid adaptation, energy consumption, ATPase, dental caries

**INTRODUCTION**

The mutans streptococci, which are thought to play a key role in the development of dental caries (Hamada & Slade, 1980; Loesche, 1986), are distinguished from other oral streptococci by their ability to grow at the low pH at which teeth enamel erodes (Harper & Loesche, 1984; McDermid et al., 1986; Bowden & Hamilton, 1987). This growth characteristic (acid tolerance) is thought to serve as a selective advantage over other micro-organisms in acidic, caries-active dental plaques, and hence can be regarded as an important factor in their cariogenicity.

Several characteristic changes have so far been reported for mutans streptococci grown under acidic environments, including a decrease in the molar glucose yield in growth (Hamilton, 1986), an increase in ATPase activity (Bender et al., 1986; Hamilton, 1991; Sturr & Marquis, 1992; Dashper & Reynolds, 1992) and a decrease in the \(\text{H}^+\) permeability of the cell membrane (Bender et al., 1986; Hamilton, 1991). The decrease in molar yield has been ascribed to a change in the glucose fermentation from a mixed-acid, to a homolactic type (Fig. 1), resulting in a decrease in the stoichiometric ratio of the ATP generated to the glucose utilized (Hamilton, 1986; Iwami et al., 1992). No appropriate experiment has yet been described, however, which has provided the means to quantify
Fig. 1. Fermentation of glucose by mutans streptococci. Under anaerobic conditions, glucose is catabolized into either a mixture of formate and acetate plus ethanol, or lactic acid. (Carlsson & Griffith, 1974).

growth energetics relative to acid tolerance. Although the $F_{ATP}$-type of proton-translocating (H$^+$-) ATPase is reportedly present in a strain of mutans streptococci (Sutton & Marquis, 1987), it remains to be seen whether it is also present in other strains and whether the observed increase in the ATPase activity can be correlated with that of the H$^+$-ATPase itself. If so, it is of physiological importance to determine the role of ATPase in acid tolerance. In other words, it is interesting to see whether the enzyme simply regulates the intracellular pH or whether it also performs other functions.

In this study, *Streptococcus rattus*, a serotype b member of the mutans streptococci, was cultured in a strictly anaerobic glucose-limited chemostat to estimate the growth parameters as a function of environmental pH. Also, the ATPase in membrane fractions prepared from bacterial cells grown under neutral and acidic pH conditions was studied by kinetic and immunochemical means. The ability of these cells to control the intracellular pH under non-growing conditions was also investigated. The results obtained suggest that the control of this organism to acidic environments is characterized by an increased energy consumption that is not directly related to biomass production but is required for maintaining cellular homeostasis, and is somehow correlated with the induction of the membrane-bound H$^+$-ATPase.

### METHODS

#### Bacterial strain and media.
*Streptococcus rattus* FA-1 (ATCC 19645) was used throughout. Among the various mutants streptococci examined, this strain showed the least tendency to adhere to the surface of culture vessels, which is critical for quantitative studies using a chemostat. The chemostat medium contained (g l$^{-1}$): Na$_2$SO$_4$, 0.5; KH$_2$PO$_4$, 0.89; K$_2$HPO$_4$, 2.6; MgCl$_2$, 6H$_2$O, 0.4; NaCl, 1.2; NH$_4$Cl, 0.3; KCl, 0.3; CaCl$_2$, 0.11; BBL tryptase peptone, 1.0; Difco yeast extract, 1.0; resazurin, 0.001; glucose, 1.8; cysteine.HCl, 0.2; and trace element solutions (Ohta & Gottschal, 1988). Trypticase–yeast (TY) medium contained (g l$^{-1}$): KH$_2$PO$_4$, 1.0; K$_2$HPO$_4$, 4.0; NaCl, 2.0; MgSO$_4$, 7H$_2$O, 0.25; BBL trypptide peptone, 20.0; Difco yeast extract, 5.0; glucose, 2.0. This medium was supplemented with agar (15.0 g l$^{-1}$) to prepare plates for checking the purity of cultures.

#### Culture conditions.
An anaerobic chemostat with a 430 ml working volume was operated at 37 °C as described by Ohra & Gottschal (1988). Cultures were started by inoculating a portion of overnight culture in TY medium, and the pH was initially maintained at pH 7.0 by the automatic addition of 2 M NaOH or 2 M HCl. The OD$_{660}$ was measured every 12 h in an LKB Novaspec 449 spectrophotometer until a steady state was established. The pH was then lowered at intervals of 0.2 to 4.8 and the establishment of the steady state of each pH was confirmed in the same manner. To repeat the steady state experiment at each pH, the chemostat run was either started again from pH 7.0 or brought back stepwise to pH 7.0. Culture samples were examined daily by microscopy or by spreading on TY-agar plates.

#### Chemical analysis and calculations.
Glucose was determined using a commercial assay kit (F-kit glucose, Boehringer-Mannheim). The end products of fermentation were analysed by gas-liquid chromatography as described previously (Ohta et al., 1989). The protein concentration was measured by the Lowry method using bovine serum albumin as the standard. The specific rate of ATP generation ($q_{ATP}$, mmol (g dry wt)$^{-1}$ h$^{-1}$) was then estimated by the equation, $q_{ATP} = q_{glucose} + q_{acetate}$ (Fig. 1), where $q_{glucose}$ and $q_{acetate}$ are, respectively, the specific rates of glucose consumption and acetate production [mmol glucose consumed or acetate formed (g dry wt)$^{-1}$ h$^{-1}$]. The efficiency of ATP generation in glucose catabolism (ATP-Eff, molar ratio of ATP formed to glucose fermented) is given by the ratio of $q_{ATP}$ to $q_{glucose}$ and the apparent growth yield for the amount of ATP formed [$Y_{ATP}$, g dry wt (mol ATP formed)$^{-1}$] by the ratio of the specific growth rate ($\mu$) to $q_{ATP}$ where $\mu$ is numerically equal to the dilution rate ($D$) under steady-state conditions. The maintenance coefficient ($m$) and the maximum yield growth ($Y_{max}$) for both the glucose fermented and the ATP generated were estimated graphically by plotting the corresponding $q$ values against $D$ with the relationship, $g = D/Y_{max} + m$ (Pirt, 1965).

#### Dry weight.
Bacterial cells were collected by centrifugate at 10000 g for 20 min and washed three times with 10 mM Tris/HCl (pH 7.5) containing 5 mM MgCl$_2$ (TM buffer). The washed cells were suspended in the same buffer, collected on weighed nitrocellulose membranes of 0.2 μm pore size and dried at 105 °C. No significant variation was found in the correlation coefficient of the cell dry weight at OD$_{660}$ for cells grown under various conditions. Thus, the averaged coefficient [0.503 ± 0.014 mg dry wt ml$^{-1}$ OD$_{660}$] (mean ± SEM, n = 8) was used to determine the cell density of cultures and washed cell suspensions.

#### Preparation of cell membranes.
The temperature was maintained below 4 °C throughout the following procedure. Washed cells suspended in TM buffer were passed several times through a high-pressure cell disrupter (Minilab, Rannie) operated at 8 × 10$^4$ kPa. The cell extract (the supernatant after centrifugation at 30000 g for 15 min) was further centrifuged at 100000 g for 60 min. The pelleted cell membrane was washed twice with and finally suspended in the TM buffer at a concentration of 10 mg protein ml$^{-1}$.

#### Assay for ATPase activity.
ATPase activity was measured at 25 °C in 10 mM MOPS containing 2 mM MgCl$_2$ and...
150 mM KCl together with an appropriate amount of the cell membrane preparation. The pH was adjusted with NaOH. The reaction was started by adding ATP (5–300 μM; Sigma) and stopped after 3 min with perchloric acid (final concentration, 0·3 M); the amount of phosphate released was measured by a modified Malachite Green method (Kodama et al., 1986). Data were analysed by fitting to the Michaelis–Menten equation using $V_{\text{max}}$ and $K_m$ as adjustable parameters.

**Immunoblotting.** Discontinuous SDS-PAGE was performed in a 10% (w/v) separating gel as described by Laemmli (1970), and stained with silver nitrate. The proteins were electrophoretically transferred to polyvinylidene difluoride membranes (Bio-Rad) by the method described by Towbin et al. (1979). Reaction with antisera against the H1-ATPase protein components of *Escherichia coli* and the thermophilic bacterium PS3 was detected by visualizing with swine anti-rabbit immunoglobulin G conjugated to peroxidase together with 4-methoxy-1-naphthol and hydrogen peroxide.

**Intracellular pH.** Washed cells were incubated at 25°C in 10 mM MES containing 20 mM glucose, 10 mM MgCl2, 150 mM KCl and 20 μM [14C]acetylsalicylate (3·7 kBq ml−1; New England Nuclear) with and without carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP, 80 μM; Sigma). After a 10 min incubation during which the pH was maintained at a constant value between 5·0 and 7·5 by the automatic addition of 0·1 M NaOH, duplicate samples were centrifuged through silicone oil (1:1·7 mixture of Dow Corning fluid 550 and 510) at 8000 $g$ for 5 min as described by Kashket & Barker (1977). The radioactivity in the aqueous phase and the cell pellet was measured using a liquid scintillation counter. The inter- and intracellular water volumes in the pellet were estimated in the same manner by incubating the cells with 3H2O (29·6 kBq ml−1; New England Nuclear) instead of glucose and 1-naphthol and hydrogen peroxide.

The limiting factor was not glucose, and the steady state was not established.

**RESULTS**

**Growth and energy metabolism**

*S. rattus* was grown in an anaerobic glucose-limited chemostat at a fixed dilution rate of 0·10 or 0·09 h−1 over the pH range from 7·0 to 4·8. A steady state was established at each pH between 7·0 and 5·0 but not at pH below 4·8 (see below). The growth parameters for these are given in Table 1.

With lowering of pH, the molar glucose yield ($Y_{\text{glucose}},\text{g dry wt (mol glucose)}^{-1}$) first decreased gradually, then rather sharply at a pH below 5·6. As the pH was further lowered below 5·0, glucose became detectable in the culture, so it was no longer glucose-limited at the dilution rate used.

The fermentation end products in the culture were determined at the steady state of each pH (Table 1). The main fermentation products at pH between 7·0 and 5·8 were formate, acetate and ethanol, but little lactate was produced. Thus, glucose underwent a mixed-acid fermentation. At a pH below 5·6, however, lactate production became noticeable, and at pH 5·2 exceeded the formate production accompanying the decrease in mixed-acid fermentation products.

The specific rate of ATP generation ($q_{\text{ATP}}$), the efficiency of ATP generation in glucose catabolism or stoichiometry of ATP generated over glucose fermented [ATP-Eff], and the apparent growth yield with respect to the amount of intracellular water spaces by the Henderson–Hasselbalch equation (Rottenberg, 1979).

**Table 1.** Specific rates of glucose utilization and of product formation expressed in anaerobic glucose-limited chemostat cultures of *S. rattus* growing at various pH values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Growth pH:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7·0</td>
</tr>
<tr>
<td>Dilution rate (h−1)</td>
<td>0·10</td>
</tr>
<tr>
<td>Yield of cells (mg dry wt ml−1)</td>
<td>0·396</td>
</tr>
<tr>
<td>Residual glucose (mM)</td>
<td>0</td>
</tr>
<tr>
<td>$Y_{\text{glucose}}$ (g cells mol−1)</td>
<td>37·3</td>
</tr>
<tr>
<td>$q$ (mmol (g cells)−1 h−1)</td>
<td>2·68</td>
</tr>
<tr>
<td>Glucose</td>
<td>2·71</td>
</tr>
<tr>
<td>Acetate</td>
<td>1·46</td>
</tr>
<tr>
<td>Ethanol</td>
<td>4·80</td>
</tr>
<tr>
<td>Formate</td>
<td>0·14</td>
</tr>
<tr>
<td>Lactate</td>
<td>8·07</td>
</tr>
<tr>
<td>ATP</td>
<td>3·01</td>
</tr>
<tr>
<td>ATP-Eff</td>
<td>12·4</td>
</tr>
<tr>
<td>C-recovery (%)</td>
<td>84</td>
</tr>
</tbody>
</table>

* The limiting factor was not glucose, and the steady state was not established.
Maximum growth yield and maintenance coefficients

From the relationship $1/Y = 1/Y_{\text{max}} + m/\mu$ for chemostat cultures in general (Pirt, 1965), the lowered apparent $Y$ values under acidic conditions can be explained by either a decrease in $Y_{\text{max}}$ or an increase in $m$, or by both. To estimate these parameters, the chemostat was operated at $D$ values between 0.05 and 0.20 h$^{-1}$ at pH 7.0, 6.2 and 5.0. Under these conditions, steady states were established and no residual glucose was detected. At pH 7.0 and 6.2, mixed-acid fermentation prevailed at dilution rates between 0.05 and 0.20 h$^{-1}$. At pH 5.0, however, it was changed by lactic fermentation, the extent of which increased with the dilution rate (Fig. 2).

$D$ was plotted against $q_{\text{glucose}}$ (Fig. 3) and $q_{\text{ATP}}$ (Fig. 4). Irrespective of pH, both plots gave straight lines, from which the corresponding values of $Y_{\text{max}}$ and $m$ were estimated (Table 2). When the pH was lowered from 7.0 to 5.0, there was a marked decrease in $Y_{\text{max}}(\text{glucose})$ whereas the pH change did not cause a significant variation in $Y_{\text{max}}(\text{ATP})$. This suggests that environmental acidification has no influence on the maximum efficiency of ATP usage in biomass formation. The decrease in $Y_{\text{max}}(\text{glucose})$ can be hence ascribed to the decrease in the efficiency of ATP generation in glucose catabolism. There was a good agreement between the ATP-Eff values estimated as the ratio of $Y_{\text{max}}(\text{glucose})$ to $Y_{\text{max}}(\text{ATP})$, and those obtained from the fixed $D$ chemostat cultures at the corresponding pH values (Table 1). There was a fivefold increase in $m_{\text{glucose}}$ and a threefold increase in $m_{\text{ATP}}$ when the pH was lowered from 7.0 to 5.0. Thus, apart from the decrease in ATP-Eff, more energy (ATP) is consumed for the maintenance under low pH conditions.
increase, the most likely is the energy consumption 
D, 
Of several possible sources responsible for the 
required to generate and maintain the concentration 
Membrane ATPase activity 

\[ m_{\text{ATP}} \] 

Dicyclohexylcarbodiimide (DCCL), 0.5 and 1.0 mM, Sigma), a specific inhibitor of 
other than the specific ATPases.

Table 2. Maximum growth yield and maintenance coefficient values of anaerobic glucose-limited chemostat cultures of S. rattus growing at various pH values

Values are mean ± SEM of at least five determinations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>7.0</th>
<th>6.2</th>
<th>5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>glucose</td>
<td>23.6 (±0.4) D + 0.16 (±0.05)</td>
<td>25.0 (±0.2) D + 0.36 (±0.03)</td>
<td>30.6 (±1.3) D + 0.80 (±0.12)</td>
</tr>
<tr>
<td>ATP</td>
<td>67.4 (±1.4) D + 0.63 (±0.17)</td>
<td>68.1 (±1.0) D + 1.32 (±0.14)</td>
<td>66.0 (±2.2) D + 2.08 (±0.20)</td>
</tr>
<tr>
<td>Y_{max(glu)}/mol}</td>
<td>42.4 ± 0.7</td>
<td>39.9 ± 0.4</td>
<td>32.7 ± 1.4</td>
</tr>
<tr>
<td>Y_{max(ADP)} (g cells mol⁻¹)</td>
<td>14.8 ± 0.3</td>
<td>14.7 ± 0.2</td>
<td>15.2 ± 0.5</td>
</tr>
<tr>
<td>m_{ATP} [mmol (g cells⁻¹ h⁻¹)]</td>
<td>0.16 ± 0.05</td>
<td>0.36 ± 0.03</td>
<td>0.80 ± 0.12</td>
</tr>
<tr>
<td>m_{ATP} [mmol (g cells⁻¹ h⁻¹)]</td>
<td>0.63 ± 0.17</td>
<td>1.32 ± 0.14</td>
<td>2.08 ± 0.20</td>
</tr>
<tr>
<td>ATP-Eff</td>
<td>2.86</td>
<td>2.71</td>
<td>2.15</td>
</tr>
</tbody>
</table>

D, dilution rate (equivalent to growth rate).

Membrane ATPase activity

Of several possible sources responsible for the \( m_{\text{ATP}} \) increase, the most likely is the energy consumption required to generate and maintain the concentration gradient of solutes across the cell membrane. The immediate, if not sole, entity that underlies membrane transport in fermentative bacteria is the \( \text{H}^+ \)-ATPase that extrudes protons out of the cytoplasm to generate the proton-motive force across the membrane. Thus, the membrane-bound ATPase was examined by kinetic and immunochemical methods to determine the involvement of the \( \text{H}^+ \)-ATPase in S. rattus. The membrane fraction was prepared from cells grown in the chemostat at pH 5.0 (acid-adapted cells) and at pH 7.0 (unadapted cells). There was no systematic variation in the recovery of membrane proteins for either of these types of cells or between culture batches, so we assumed the ATPase activity of membrane fractions to be a measure of that for the whole cell. In measuring the activity, the ATP concentration was not more than 300 µM, to minimize hydrolysis by enzymes other than the specific ATPases.

The activity of the adapted cells was significantly higher than that of the unadapted cells (see 'FCCP(-)' in Table 3). This 10-fold increase was accompanied by a threefold increase in \( K_m \), which rather suggests a qualitative change in the ATP splitting system. Dicyclohexylcarbodiimide (DCCD, 0.5 and 1.0 mM, Sigma), a specific inhibitor of bacterial \( \text{H}^+ \)-ATPases, inhibited the adapted-cell membrane by 50 and 75% respectively, but not the unadapted-cell membrane. The activity was also measured in the presence of a protonophore, FCCP (20 µM). FCCP strongly enhanced the activity of the adapted-cell membranes without a significant change of \( K_m \). A mixture of valinomycin (2.3 µM) and nigericin (1.7 µM) acting as a proton conductor in the presence of \( K^+ \) induced a similar enhancement. The FCCP-sensitive ATPase activity was maximal at pH 6.2 (Fig. 5). In contrast, the protonophore did not enhance the unadapted-cell membranes. These results indicate that under acidic conditions, the organism synthesizes the ATPase that functions as an electrogenic proton-pump (see Discussion).

We also compared the ATPase protein components with those of other bacteria. The membrane fractions from both adapted and unadapted cells were resolved by SDS-PAGE and immunoblotted using antisera against the \( \alpha \)-subunit of Escherichia coli F_{ATPase} and the \( \beta \)-subunit of the homologous enzyme from a thermophilic bacterium Ps3 (Fig. 6). The adapted-cell membranes, an immunoreactive protein band was seen at the position of 58 kDa with the anti-\( \alpha \) serum and another band at 52 kDa with the anti-\( \beta \) serum. The protein bands corresponding to the immunoreactivity were seen by silver staining. By con-

Table 3. ATPase activities of S. rattus membrane cultured in an anaerobic chemostat at different pH values (assay at pH 6.8) in the presence (+) and absence (−) of the protonophore FCCP

Values for ATPase activities are mean ± SEM of three independent determinations except for those at pH 7.0 in the presence of FCCP, which are means and ranges for two determinations.

<table>
<thead>
<tr>
<th>Growth pH</th>
<th>FCCP (−)</th>
<th>FCCP (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( K_m ) (mM)</td>
<td>( V_{max} ) (nmol ( \text{min}^{-1} ) ( \text{mg}^{-1} ))</td>
</tr>
<tr>
<td>7.0</td>
<td>0.06 ± 0.02</td>
<td>22 ± 5</td>
</tr>
<tr>
<td>5.0</td>
<td>0.17 ± 0.02</td>
<td>179 ± 41</td>
</tr>
</tbody>
</table>
Fig. 5. Membrane ATPase activity of *S. rattus* cells grown at pH 5.0 in anaerobic glucose-limited chemostat culture. Activities were measured with 0.3 mM ATP in the presence (○) and absence (●) of FCCP.

**Fig. 6.** SDS-PAGE of the cell membrane fraction of *S. rattus* in anaerobic glucose-limited chemostat cultures at pH 5.0 (lane 1) and 7.0 (lane 2). (a) silver stain; (b, c) Western blots with antisera against the α-subunit of the *E. coli* F,Fo-ATPase (b) and the β-subunit from the thermophilic bacterium PS3 (c). Arrows indicate the 58 kDa and 52 kDa proteins.

... contrast, the two immunoreactive bands were undetectable in the unadapted-cell membranes.

**Intracellular pH control**

In view of the marked difference in the amount of H+-ATPase between the adapted and unadapted cells, their ability to control the intracellular pH was compared by incubating them with glucose under non-growth conditions (Fig. 7). Over the pH range examined, both of these types of cells established and maintained the ΔpH, with the cytoplasm being alkaline relative to the extracellular fluid. The ΔpH value increased as the pH was lowered, reaching 1.2 at pH 5. These results suggest that despite induction of the H+-ATPase under acid conditions this enzyme does not appear to play any apparent role in cytoplasmic pH homeostasis.

**DISCUSSION**

The results described here indicate that the environmental acidification causes *S. rattus* to increase the rate of growth-unrelated energy consumption (the maintenance coefficient) and to induce membrane H+-ATPase. In addition, we have confirmed earlier studies suggesting a metabolic shift from mixed-acid to homolactic fermentation in mutans streptococci (Hamilton, 1986; Iwami et al., 1992).

Taking into account the procedures of membrane preparation, the kinetic and immunochemical properties and sensitivity to DCCD and protonophores, there is little doubt as to the presence in *S. rattus* of an F1,Fo-type of ATPase similar to that found in other bacteria. In fermentatives, the enzyme generates an inward proton-motive force (Δp) across the cell membrane by splitting ATP (Nicholls, 1982). When disrupted by repetitive passage through a released-compression device as described here, the cell membrane would be fragmented to generate inside out vesicles (*V*io) and right-side out vesicles (*V*ro) together with non-vesicular fragments (NF). Although the F1 (ATP splitting) portion is considered accessible to the substrate ATP in the enzyme bound to both *V*io and NF, the *V*io-bound activity would be restrained by Δp directed outwardly, from the vesicular lumen, which should be generated by the enzyme. In addition, the *V*ro-bound enzyme would have no access to ATP. Hence, the activity of the membrane fraction as a
whole should be very low if measured by conventional means, and the observed enhancement by FCCP could thus be a reflection of the dissipation of $\Delta p$. This interpretation is consistent with a preliminary observation that Triton X-100 markedly (threefold) enhanced the activity, since the membrane should be rendered permeable not only to protons but also to ATP. Triton X-100 did not enhance the membranes of unadapted cells.

We found that even the unadapted cells can keep the cytoplasm alkaline relative to the acidic environment as effectively as the adapted cells. Among several explanations for this apparent paradox, the simplest is probably that the organism is equipped with a constitutive pH controlling system that is formed irrespective of the environmental pH. Alternatively, the unadapted cells can maintain $\Delta p$H by operating a trace level of H$^+$-ATPase when simply fermenting glucose under non-growing conditions. In either of these situations, the induction of H$^+$-ATPase would be optional for cells to overcome the adversity to growth caused by acidic conditions.

Besides the decrease in the efficiency of ATP generation (ATP-Eff), an important aspect of the fermentation shift is that, in effect, the stoichiometric ratio of the total acid produced to the glucose utilized decreases from 3 (2 formic acids +1 acetic acid) in the mixed acid fermentation, to 2 in the lactic fermentation. This change is reminiscent of earlier observations that fermentative bacteria convert acid products to neutral compounds at acidic pH as does Clostridium acetobutylicum, which produces butanol from butyrate (Gottschalk & Morris, 1981; Gottschalk, 1988). The shift from acetic acid (pKa, 4.75) to lactic acid (pKa, 3.86) has been regarded as a mechanism which averts the toxic, uncoupling function of the former since its undissociated form, which is lipophilic, and probably membrane-permeable, dominates at pH below 5.0 (Gottschalk, 1988). In our view, however, these metabolic shifts may be of adaptive importance for the organisms to maintain a constant intracellular pH during growth on sugars even by sacrificing the efficiency of ATP generation, since they retard environmental acidification and hence reduce the burden on the organisms.

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