Ammonium/Urea-Dependent Generation of a Proton Electrochemical Potential and Synthesis of ATP in *Bacillus pasteurii*

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The influence of ammonium and urea on the components of the proton electrochemical potential (Δp) and de novo synthesis of ATP was studied with *Bacillus pasteurii* ATCC 11859. In washed cells grown at high urea concentrations, a Δp of −56 ± 29 mV, consisting of a membrane potential (Δψ) of −228 ± 21 mV and of a transmembranar pH gradient (ΔpH) equivalent to 172 ± 38 mV, was measured. These cells contained only low amounts of potassium, and the addition of ammonium caused an immediate net decrease of both Δψ and ΔpH, resulting in a net increase of Δp of about 49 mV and de novo synthesis of ATP. Addition of urrea and its subsequent hydrolysis to ammonium by the cytosolic urease also caused an increase of Δp and ATP synthesis; a net initial increase of Δψ, accompanied by a slower decrease of ΔpH in this case, was observed. Cells grown at low concentrations of urea contained high amounts of potassium and maintained a Δp of −113 ± 26 mV, with a Δψ of −228 ± 22 mV and a ΔpH equivalent to 115 ± 20 mV. Addition of ammonium to such cells resulted in the net decrease of Δψ and ΔpH without a net increase in Δp or synthesis of ATP, whereas urea caused an increase of Δp and de novo synthesis of ATP, mainly because of a net increase of Δψ. The data reported in this work suggest that the ATP-generating system is coupled to urea hydrolysis via both an alkalization of the cytoplasm by the ammonium generated in the urease reaction and a net increase of Δψ that is probably due to an efflux of ammonium ions. Furthermore, the findings of this study show that potassium ions are involved in the regulation of the intracellular pH and that ammonium ions may functionally replace potassium to a certain extent in reducing the membrane potential and alkalizing the cytoplasm.

**MATERIALS AND METHODS**

Microorganism and culture conditions. *B. pasteurii* ATCC 11859 was grown aerobically at 28°C in a medium containing 20.0 g of yeast extract per liter and the amount of urea indicated in each experiment; the pH was adjusted to 9.0 with NaOH prior to autoclaving. The cells were harvested from early exponential growth and washed twice with a buffer containing 20 mM CAPSO (3-cyclohexylamino-2-hydroxy-1-propanesulfonic acid), 20 mM NaCl, and 100 μM of chlor amphenicol mL⁻¹ at pH 9.0 (buffer A).

**Measurement of ΔpH and Δψ.** Washed cells were resuspended in buffer A to give a cell density of between 0.2 and 0.3 mg of protein mL⁻¹; 1 mL of the suspension was incubated at 25°C for 5 min with stirring, before the radioisobolophil probe was added. Δψ was determined by addition of 2.5 μM [phenyl-4,5]tetraphenylphosphonium bromide (phenyl-4,5-[C]TPP) (1.85 × 10⁻¹⁰ Bq; 0.74 × 10⁻⁸ Bq mmol⁻¹) and centrifugation in Eppendorf tubes (3 min at 12,000 × g of 250 μL of the cell suspension after 2, 8, and 15 min through a 200-mL silicon oil layer (a mixture of 6 volumes of silicon oil AR 200 [d = 1.04; Serva, Heidelberg, Germany] and 1 volume of silicon oil DC 200 [d = 0.96; Serva]). One hundred microliters of the cell-free supernatant was added to 0.4 mL of 0.4 N NaOH before 5.5 mL of scintillation cocktail (Quicksafe A; Zinsser Analytic) was added. After the remainder of the supernatant and most of the oil were carefully aspirated, the tip of the Eppendorf tube containing the cell pellet was cut off and the cell pellet was added to 0.4 mL of 0.4 NaOH after 24 h of incubation at 30°C, 5.5 mL of scintillation cocktail was added and radioactivity was determined by liquid scintillation counting. Since the apparent Δψ determined by the measured of the distribution of [phenyl-4,5-[C]TPP may depend on the [phenyl-4,5-[C]TPP concentration used (2, 9), the concentration of [phenyl-4,5-[C]TPP was varied between 0.5 and 10.0 μM in one set of experiments; no significant differences in the values of Δψ determined after a 15-min incubation with various concentrations of [phenyl-4,5-[C]TPP were observed (results not shown). ΔpH (z ΔpH, with z = 59 mV) was determined from the distribution of 2.5 μM [¹⁴C]methylamine (1.85 × 10⁻¹⁰ Bq; 2.07 × 10⁻⁸ Bq mmol⁻¹) after 2, 8, and 15 min of incubation with this compound, as described above. This technique could be used without interference from an ammonium transport system, since *B. pasteurii* lacks such a system (19). Controls for energy-independent probe binding were performed for each assay of both the determination of the membrane potential and the intracellular pH by preincubating cells with 20 μM gramicidin D for 15 min prior to the addition of the radioisobolophil compounds; the values for Δψ were corrected for nonspecific binding by the exponential mean model according to

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TABLE 1. Influence of monovalent inorganic cations and urea on B. pasteurii grown at different urea concentrationsa

<table>
<thead>
<tr>
<th>Compound added (concentracon)</th>
<th>Electrochemical parameter or ATP contentb in cells grown at urea concen</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>15 mM</td>
</tr>
<tr>
<td></td>
<td>Δφ (mV)</td>
</tr>
<tr>
<td>None</td>
<td>−228 ± 22</td>
</tr>
<tr>
<td>None</td>
<td>−215 ± 21</td>
</tr>
<tr>
<td>Urea (50 mM)</td>
<td>−245 ± 22</td>
</tr>
<tr>
<td>NH₄Cl (50 mM)b</td>
<td>−170 ± 20</td>
</tr>
<tr>
<td>KCl (50 mM)</td>
<td>−205 ± 15</td>
</tr>
<tr>
<td>NaCl (50 mM)</td>
<td>−228 ± 12</td>
</tr>
</tbody>
</table>

a Values for electrochemical parameters and ATP contents were determined 2 and 3 min, respectively, after the addition of the indicated compunds to cells harvested from exponential growth, washed twice, and resuspended in buffer A at 30°C, except as indicated.

b All values are means ± standard errors of the means for three independent experiments.

<table>
<thead>
<tr>
<th></th>
<th>ΔpH (mV)</th>
<th>Δp (mV)</th>
<th>ATP (mM)</th>
</tr>
</thead>
</table>

The values for Δψ and ΔpH were determined both in cells washed twice and suspended in buffer A and in cells washed twice with buffer A and resuspended in the supernatant of the first centrifugation step (growth medium). The data in Table 1 indicate that the intracellular pH of cells resuspended in buffer A was significantly more alkaline than that of cells resuspended in growth medium; this effect was more pronounced in cells grown at a high urea concentration than in cells grown at a low concentration of this compound (Table 1). The values for Δψ were identical in cells grown at different urea concentrations and resuspended in buffer A; significantly lower values of Δψ were measured in cells resuspended in the growth medium, especially after growth at a high urea concentration (Table 1). The determination of intracellular pH with permeant amines at low values of ΔpH is known to be unreliable; however these data were confirmed in experiments in which the intracellular pH was estimated by measuring the fluorescence change of 9-aminoacridine (results not shown). The findings suggested that compounds of the growth medium were necessary for the maintenance of an alkaline pH in B. pasteurii, especially when grown at a high urea concentration. Since the only difference between the media was the urea concentration used for growth and since urea was completely hydrolyzed by the time the cells were harvested, the influences of both urea and ammonium on ΔpH and Δψ were investigated. The experiments were performed at pH 9.0, near the pK of ammonium (9.25), and no increase or decrease of the extracellular pH of more than 0.1 pH unit was measurable during the assay periods in these studies.

The addition of 50 mM ammonium to cells grown at a high concentration of urea resulted in immediate alkalization of the cytoplasm accompanied by a net decrease of Δψ and resulted in a net increase of ΔpH. These changes were insignificant when ammonium was added at concentrations between 2 and 10 mM (results not shown); the addition of up to 300 mM ammonium had the same effects on Δψ and ΔpH as shown in Fig. 1. In cells grown at a low urea concentration, the addition of ammonium also resulted in a net decrease of Δψ and an alkalization of the cytoplasm; however, no significant net increase of ΔpH was measured (Fig. 1). The addition of urea at concentrations between 25 and 200 mM resulted in an increase of ΔpH in cells grown at both high and low urea concentrations (Fig. 2); at urea concentrations between 1 and 5 mM, almost no effect on ΔpH was observed (results not shown). Measurements of the change of fluorescence of rhodamine 6G indicated a rapid initial increase of Δψ (within less than 15 s after the addition of urea) under these conditions (results not shown). These findings were confirmed by measurements of the distribution of the lipophilic cation [phenyl-14C]tetraphenylphosphonium over the cell membrane: an initial net increase of Δψ, accompanied by a slow increase of intracellular pH, resulted in an overall increase of ΔpH (Fig. 2). In order to determine the specificities of ammonium and urea...
in affecting the electrochemical parameters in *B. pasteurii*, the influences of both other monovalent cations and permeant amines on $\Delta\psi$ and $\Delta pH$ were studied. The addition of methyamine and ethanalamine resulted in an increase of the intracellular pH, a net decrease of $\Delta\psi$, and a net increase of $\Delta p$ similar to those observed upon the addition of ammonia (results not shown); of the other monovalent cations tested, only potassium significantly affected the electrochemical parameters, decreasing $\Delta\psi$ and increasing the intracellular pH (Table 1).

**Intracellular ATP concentration and ATPase activity.** Both urea and ammonium profoundly affected $\Delta p$ in *B. pasteurii*, and the influence of these compounds on the ATP content was subsequently studied in cells grown at different concentrations of urea. The ATP content of *B. pasteurii* obviously correlated with the determined electrochemical parameters (Table 1); while low intracellular ATP concentrations were observed in resting cells grown at high urea concentrations, about a 10-fold increase in concentration was measured in cells grown at low concentrations of urea. ATP synthesis was strongly stimulated by urea and ammonium in cells grown at high urea concentrations but was only slightly stimulated in cells grown at low urea concentrations (Table 1); the addition of other permeant amines (methylamine and ethanalamine) had the same effects on ATP synthesis as those observed upon addition of ammonia (results not shown). This urea- and ammonium-mediated stimulation of ATP synthesis was observed in a range between pH 7.5 and 10.5 in 50 mM Tris-HCl buffer A, and 50 mM glycine-NaOH (results not shown); incubation of the cells at a pH below 7.5 or above 11 resulted in a rapid loss of viability in both the presence and absence of potassium and/or sodium ions (results not shown). Acetohydroxamic acid, a potent inhibitor of bacterial ureases, almost completely abolished the urea-stimulated but not the ammonium-stimulated ATP synthesis (Table 2). The inhibition of ATPase by DCCD and the reduction of $\Delta\psi$ by the addition of KCl and valinomycin severely inhibited both the urea- and ammonium-stimulated synthesis of ATP (results not shown); the protonophore CCCP had only a weak inhibitory effect (Table 2). The presence of the ionophore gramicidin completely inhibited de novo ATP synthesis; contrary to the results with CCCP, the $\Delta p$H was rapidly abolished by gramicidin, as observed in experiments measuring the fluorescence quench of 9-aminoacridine (Fig. 3).

The time course of ammonium/urea-stimulated ATP synthesis was studied in cells grown at high concentrations of urea. Despite the immediate increase of $\Delta p$ upon the addition of

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**FIG. 1.** Effect of ammonium on components of $\Delta p$ in *B. pasteurii*. Cells grown at urea concentrations of 15 mM (open symbols) or 300 mM (closed symbols) were harvested from exponential growth, washed twice, and incubated in buffer A at 30°C. The arrow indicates the addition of ammonium chloride at a final concentration of 50 mM.

**FIG. 2.** Effect of urea on components of $\Delta p$ in *B. pasteurii*. Cells grown at urea concentrations of 15 mM (open symbols) or 300 mM (closed symbols) were harvested from exponential growth, washed twice, and incubated in buffer A at 30°C. The arrow indicates the addition of urea at a final concentration of 50 mM.
urea (Table 1), the urea-mediated stimulation of ATP synthesis started with a lag after the addition of urea, when the internal pH had risen above 7.0 (Fig. 4). The addition of ammonium led both to an immediate rise of the cytoplasmic pH and to de novo synthesis of ATP (Fig. 4); the simultaneous addition of both ammonium and urea resulted in both the rapid alkalinization of the cytoplasm and the immediate onset of ATP synthesis (Fig. 4).

The influence of ammonium and urea on the ATPase activity was studied in more detail in order to differentiate between a direct stimulation of ATPase by ammonium, as it has been described for the enzyme of a facultatively anaerobic alkali-philic strain Ep01 (*A. xylanus*; 14), and an indirect stimulation via an alkalinization of the cytoplasm and formation of ΔpH. In *B. pasteurii*, ATPase activity exhibited a broad pH optimum between pH 7.5 and 9.0, with only low levels of activities observed at pH 7.0 (Fig. 5), explaining the observed lag of urea-stimulated ATP synthesis despite the immediate increase of ΔpH (Table 1; Fig. 4). Urea and ammonium at concentrations between 5 and 500 mM did not stimulate ATPase activity at any pH; ammonium became inhibitory at concentrations above 250 mM (Fig. 5), concentrations that are observed in *B. pasteurii* under growth conditions (see below).

Transmembrane gradient of potassium and ammonium. Potassium is known to exert profound effects on the intracellular
TABLE 3. Intracellular and extracellular concentrations of ammonium and potassium in B. pasteurii grown at different urea concentrations

<table>
<thead>
<tr>
<th>Urea concn (mM) at which cells were grown</th>
<th>K$_{\text{in}}$ (mM)</th>
<th>K$_{\text{out}}$ (mM)</th>
<th>NH$<em>4^+</em>{\text{in}}$ (mM)</th>
<th>NH$<em>4^+</em>{\text{out}}$ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15$^a$</td>
<td>705 ± 54</td>
<td>18 ± 2</td>
<td>320 ± 45</td>
<td>45 ± 5</td>
</tr>
<tr>
<td>15$^b$</td>
<td>688 ± 41</td>
<td>ND$^c$</td>
<td>15 ± 8</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>300$^b$</td>
<td>26 ± 14</td>
<td>30 ± 2</td>
<td>620 ± 82</td>
<td>595 ± 25</td>
</tr>
<tr>
<td>300$^a$</td>
<td>22 ± 4</td>
<td>ND</td>
<td>152 ± 44</td>
<td>0.8 ± 0.2</td>
</tr>
</tbody>
</table>

$^a$ Values are means ± standard errors of the mean for three independent experiments. K$_{\text{in}}$, intracellular potassium; K$_{\text{out}}$, extracellular potassium; NH$_4^+_{\text{in}}$, intracellular ammonium; NH$_4^+_{\text{out}}$, extracellular ammonium.
$^b$ Cells centrifuged once and resuspended in the supernatant.
$^c$ Cells washed twice with and resuspended in buffer A.

DISCUSSION

The results presented in this work clearly show that both ammonium and urea exhibit profound effects on electrochemical parameters and ATP synthesis in B. pasteurii, especially when the cells were grown under optimal conditions, i.e., at a high concentration of urea. High ammonium concentrations in B. pasteurii were measured under these conditions, and washing of these cells resulted in a depletion of internal ammonium (Table 3). This resulted in unusually high values of $\Delta$F, since cells resuspended in the growth medium exhibited significantly lower values of $\Delta$F (Table 1), which are in the range of those observed in other alkaliphiles (9). Obviously intracellular ammonium ions reduce $\Delta$F and allow the cells to pump out more protons to alkalize their cytoplasm under these growth conditions, and the loss of ammonium results in an increase of $\Delta$F and decrease of the cytoplasmic pH (Table 1). The addition of ammonium or other permeant amines to such cells resulted in a net increase of $\Delta$F; this increase was due to the alkalinization of the cytoplasm, since a net decrease of $\Delta$F was observed under these conditions (Fig. 1; Table 1). These effects were observed only upon the addition of ammonium at concentrations above 20 mM, which are in the physiological range for growth of B. pasteurii. Similar observations were made for Enterococcus hirae; 5 mM ammonium had almost no effect on the cytoplasmic pH of this organism, whereas rapid alkalinization occurred upon the addition of 50 mM of this permeant amine (12). For B. pasteurii growing at high urea concentrations, ammonium is obviously necessary for a sufficient alkalinization of the cytoplasm; the observed reduction of the membrane potential upon the addition of ammonium to washed cells may result from both an influx of NH$_4^+$, directly reducing $\Delta$F, and a diffusion of NH$_3$ into the cell, resulting in the observed alkalinization of the cytoplasm and allowing an influx of protons via ATP synthetase.

The addition of urea to cells grown at a high concentration of this compound led to a net increase of $\Delta$F and an alkalinization of the cytoplasm, resulting in an increase of $\Delta$F (Fig. 2, Table 1); this increase depended on the intracellular urease activity, since it was prevented by the urease inhibitor acetohydroxamic acid (Table 2). It may be assumed that after the hydrolysis of urea, a protonation of ammonia generated by the hydrolysis of urea occurs and that this is followed by its efflux. A concentration difference of ammonium (inside the cells/outside the cells) of up to 30-fold was observed after the addition of 50 mM urea to resting cells of B. pasteurii (Table 4), a difference which corresponds to a $\Delta$F of 85 mV for ammonium, calculated as described previously (20). The concentration gradient of ammonium may act as a driving force for such an efflux; when appearing in the extracellular space, a significant amount of the excreted ammonium is deprotonated at an extracellular pH of 9.0; therefore, the NH$_4^+$ concentration difference would even be higher. However, the highest ammonium concentration gradients, of approximately 150-fold, were observed in washed cells of B. pasteurii prior to the addition of extracellular urea (Table 4); under these conditions, the efflux of ammonium may be prevented at least in part by the $\Delta$F of $\sim$228 mV, retaining the positively charged NH$_4^+$ in the cytoplasm. At an intracellular pH of 6.1 observed under these conditions (Fig. 5), a reduction of $\Delta$F via an ATP synthase-mediated proton flux and simultaneous synthesis of ATP is inhibited. Upon addition of urea, the intracellular pH rapidly rises about 0.9 units to pH 7.0 (Table 4), and the influx of protons via the ATP synthetase, generating ATP and reducing $\Delta$F, may support an efflux of ammonium ions. Future studies will have to show if an efflux system for ammonium ions is involved in this process; the presence of such a system has been discussed for U. urealyticum, for which a net increase of $\Delta$F of about 20 mV has been observed upon the addition of urea (28). This effect of urea was by far more pronounced in B. pasteurii than in U. urealyticum, both with respect to the increase of $\Delta$F and the pH range at which stimulation of ATP synthesis was observed. While the stimulation of ATP synthesis occurred in a narrow pH range around 6.9 in U. urealyticum, ATP synthesis in B. pasteurii was enhanced within the pH range of 7.5 to 9.5. Furthermore, simultaneous addition of

TABLE 4. Effects of urea on the intracellular and extracellular ammonium concentrations, cytoplasmic pH, and ATP content in B. pasteurii

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>NH$<em>4^+</em>{\text{in}}$ (mM)</th>
<th>NH$<em>4^+</em>{\text{out}}$ (mM)</th>
<th>ATP$_{\text{in}}$ (mM)</th>
<th>pH$_{\text{in}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>−5</td>
<td>142</td>
<td>0.8</td>
<td>0.58</td>
<td>6.1</td>
</tr>
<tr>
<td>−2</td>
<td>135</td>
<td>0.8</td>
<td>0.56</td>
<td>6.1</td>
</tr>
<tr>
<td>2</td>
<td>354</td>
<td>11.4</td>
<td>0.80</td>
<td>7.0</td>
</tr>
<tr>
<td>5</td>
<td>361</td>
<td>40.8</td>
<td>2.15</td>
<td>7.2</td>
</tr>
<tr>
<td>10</td>
<td>405</td>
<td>70.5</td>
<td>6.40</td>
<td>7.4</td>
</tr>
<tr>
<td>20</td>
<td>395</td>
<td>92.0</td>
<td>6.35</td>
<td>7.5</td>
</tr>
</tbody>
</table>

$^a$ Cells grown at a urea concentration of 300 mM were harvested from exponential growth, washed twice, and resuspended in buffer A at 30°C. At 0 min, 50 mM urea was added. NH$_4^+_{\text{in}}$, intracellular ammonium; NH$_4^+_{\text{out}}$, extracellular ammonium; ATP$_{\text{in}}$, intracellular ATP; pH$_{\text{in}}$, intracellular pH.
both ammonium and urea to resting cells of \textit{B. pasteurii} resulted in the immediate synthesis of ATP, which was higher than after the addition of urea or ammonium alone (Fig. 4). Ammonium has been shown to drastically inhibit the urea-stimulated synthesis of ATP (25), in \textit{U. urealyticum}, and this was ascribed to a reduction of the ammonium chemical potential by external ammonium.

As expected, ATP synthesis in \textit{B. pasteurii} was severely inhibited by DCCD, an inhibitor of \textit{F}_{0}\textit{F}_{1} ATPases (Table 2). Although very effective in inhibiting the uptake of glutamine in \textit{B. pasteurii} (10), the protonophore CCCP had only minor effects on the urea-induced ATP synthesis in this organism (Table 2), and the addition of 100 \textmu M CCCP led to almost no increase of the acidic intracellular pH (Fig. 3). The acidification of the cytoplasm in these cells may be caused by a Donnan potential; the involvement of such a Donnan potential in the acidification of the cytoplasm of the facultatively anaerobic alkaliphile strain \textit{EptiH} (15), now classified as \textit{A. xylanus} (21), has also been discussed. In such a case, the formation of \Delta\phi would depend on the proton permeability of the membrane and would primarily not be dissipated in the presence of a protonophore. Assuming that CCCP abolishes \Delta\phi (by equating \Delta\psi and \Delta\phi terms to be equal and opposite), a large flux of ammonia occurring upon the addition and hydrolysis of urea may swamp the effect of this protonophore in \textit{B. pasteurii} and permit the synthesis of ATP; a similar suggestion has been made for the urea-induced synthesis of ATP in \textit{U. urealyticum} (28). As expected, valinomycin and KCl, abolishing the membrane potential in \textit{B. pasteurii} (results not shown), and gramicidin, inducing a flux of monovalent cations across the membrane and dissipating \Delta\phi (Fig. 3), completely inhibited the urea-stimulated de novo synthesis of ATP.

In cells grown at a low concentration of urea, both a higher \Delta\phi and \Delta\phi due to a more alkaline cytoplasm and higher intracellular ATP concentrations than in cells grown at high urea concentrations were measured. The addition of ammonium did not result in de novo ATP synthesis; the acidification of the cytoplasm due to the diffusion of ammonia into the cytoplasm and its subsequent protonation to \textit{NH}_{4}^{+} was accompanied by a decrease of the membrane potential, probably due to an influx of ammonium ions, and resulted in almost no net increase of \Delta\phi. Upon the addition of urea, net increases of \Delta\phi and de novo ATP synthesis were measured in these cells, observations similar to those made for cells grown at high urea concentrations. While a lag between the addition of urea and increase of intracellular ATP in cells grown at high urea concentrations was observed, an immediate rise of the cellular ATP concentration was measured upon the addition of urea in cells grown at low concentrations of this compound (Table 1), obviously due to the more alkaline cytoplasmic pH permitting an immediate onset of ATP synthesis. This more alkaline intracellular pH in \textit{B. pasteurii} grown at a low urea concentration correlated with higher intracellular potassium concentrations compared with those of cells grown at a high urea concentration (Table 3), even after repeated washing of the cells. Potassium probably reduces the membrane potential, which allows the cell to pump out more protons, thus conserving \Delta\phi into a \Delta\phi. Such an interconversion of the components of \Delta\phi in the presence of potassium ions has been shown to occur in a number of microorganisms (1, 3), and \textit{K}^{-}-depleted cells of \textit{Streptococcus lactis} and \textit{Rhodobacter sphaeroides} were unable to regulate their cytoplasmic pHs (1, 23). In \textit{B. pasteurii}, the addition of potassium resulted in a net decrease of \Delta\phi, especially in cells grown at a high concentration of urea (Table 1); this was probably due to a rapid electrogenic \textit{K}^{+} uptake into these potassium-depleted cells (Table 3), and was accompanied by an alkalinization of the cytoplasm (Table 1). Because of their fast transmembrane diffusion and intracellular protonation, permanent amines may substitute for potassium in \textit{B. pasteurii} leading more directly to an alkalinization of the cytoplasm and an increase of \Delta\phi. This would allow the influx of protons via the ATP synthetase and lead to a reduction of the membrane potential and ATP synthesis (Fig. 1 and 4; Table 1). The direct stimulation of ATPase by ammonium, as described previously for \textit{A. xylanus} (14), was not observed in \textit{B. pasteurii}.

The results of the present study show that in \textit{B. pasteurii}, ammonium is involved in the regulation of the internal pH and replaces potassium under certain growth conditions. Furthermore, several factors are involved in the stimulation of ATP synthesis. Upon the addition of urea and/or ammonium, an alkalinization of the cytoplasm occurs that results in both an increase of \Delta\phi and an activation of ATPase, which is almost inactive at pHs below 6.8 (Fig. 5). Efflux of \textit{NH}_{4}^{+} generated by the hydrolysis of urea further leads to an increase of the membrane potential and thereby to an increase of \Delta\phi.

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REFERENCES