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REVIEW

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Mechanisms of cytoplasmic pH regulation in alkaliphilic strains of *Bacillus*

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Abstract The central challenge for extremely alkaliphilic *Bacillus* species is the need to establish and sustain a cytoplasmic pH that is over two units lower than the highly alkaline medium. Its centrality is suggested by the strong correlation between the growth rate in the upper range of pH for growth, i.e., at values above pH 10.5, and the cytoplasmic pH. The diminishing growth rate at extremely high pH values correlates better with the rise in cytoplasmic pH than with other energetic parameters. There are also general adaptations of alkaliphiles that are crucial prerequisites for pH homeostasis as well as other cell functions, i.e., the reduced basic amino acid content of proteins or segments thereof that are exposed to the medium, and there are other challenges of alkaliphily that emerge from solution of the cytoplasmic pH problem, i.e., reduction of the chemiosmotic driving force. For cells growing on glucose, strong evidence exists for the importance of acidic cell wall components, teichuronic acid and teichuronopeptides, in alkaliphily. These wall macromolecules may provide a passive barrier to ion flux. For cells growing on fermentable carbon sources, this and other passive mechanisms may have a particularly substantial role, but for cells growing on both fermentable and nonfermentable substrates, an active Na^+-dependent cycle is apparently required for alkaliphily and the alkaliphile's remarkable capacity for pH homeostasis. The active cycle involves primary establishment of an electrochemical gradient via proton extrusion, a secondary electrogenic Na^+/H^+ antiport to achieve net acidification of the cytoplasm relative to the outside pH, and mechanisms for Na^+ re-entry. Recent work in several laboratories on the critical antiporters involved in this cycle has begun to clarify the number and characteristics of the porters that support active mechanisms of pH homeostasis.

Key words Alkaliphile · pH homeostasis · Antiporters · Teichuronopeptide · Buffering capacity

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Introduction

Extremely alkaliphilic *Bacillus* species are thus far the most broadly characterized examples of bacteria that grow optimally at pH 10 and above, and even in these extremophiles, the basis for successfully meeting the central physiological challenge of growth at high external pH is incompletely resolved. Several very different sorts of mechanisms have been suggested as having a role in alkaliphily. These include passive mechanisms such as production of cell wall macromolecules that afford a special barrier to flux of relevant ions (Aono and Ohtani 1990; Ito et al. 1994; Aono et al. 1995) and elevation of cytoplasmic buffering capacity at highly alkaline growth pH (Krulwich et al. 1985a). They also include active mechanisms such as the use of Na^+/H^+ antiporters that, powered by the primary electrochemical proton gradient (Δp) that is established by respiration or the proton-translocating F_1F_O-ATPase, are able to catalyze net proton accumulation (Kitada and Horikoshi 1992; Krulwich 1995). However, there is still much work to be done on each of the proposed mechanisms, let alone the possibility that others will yet emerge.

For those mechanisms already proposed, the details of the molecules used and their modes of action and regulation are far from fully elucidated. It is not clear, for example, whether the cell wall components, cytoplasmic buffers, or antiporters used by alkaliphiles are essentially the same as those used in neutralophiles but are perhaps present in higher amounts, or whether there are special features and molecules that are required to meet the demands of alkaliphily. At least in one instance, that of the antiporters

used, there appears to be a qualitative difference, inasmuch as the antiport activities that support pH homeostasis of neutralophilic *Bacillus subtilis* at moderately alkaline pH utilize either cytoplasmic K^+ or Na^+, whereas those of alkaliphiles appear to be Na^+-specific (Krulwich et al. 1985b, 1994; and see Table 1). Moreover, the balance of importance of various mechanisms for cytoplasmic pH regulation has not been clarified. There are compelling data, from non-alkaliphilic mutant strains and studies of the antiporter-related Na^+-dependence of alkaliphily, that active mechanisms are essential (Krulwich et al. 1996; Kitada et al. 1994). However, there is also evidence that at least some of the other mechanisms are important and even essential under at least some conditions of growth (Aono and Ohtani 1994). No explicit evaluation of the contribution of different mechanisms has been conducted under any given set of growth conditions, let alone more than one. There are data suggesting that, for example, passive mechanisms may play a more substantial auxiliary role to active ones when alkaliphilic *Bacillus* species are growing on fermentative growth substrates than on nonfermentative substrates. Thus, if the balance of roles of various passive and active mechanisms is to be clarified, it will be important to transcend the tendency for the different mechanisms to be explored in different laboratories, each with its own standard growth condition and its own standard assay for pH homeostasis.

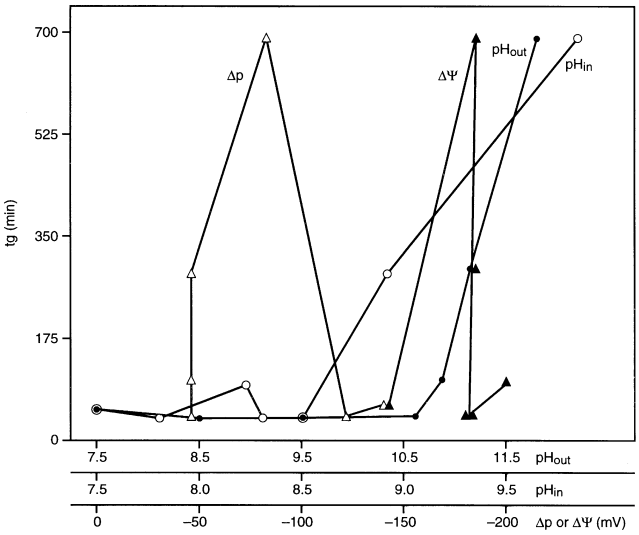

Fig. 1. The variation in magnitude of the generation time (*tg*) of alkaliphilic *Bacillus firmus* OF4 with the external pH (pH_{out}; *closed circles*), cytoplasmic pH (pH_{in}; *open circles*), electrochemical proton gradient; *open triangles* (Δp), and transmembrane electrical potential; *closed triangles* ($\Delta\Psi$). The data are replotted from those of Sturr et al. (1994) as obtained from studies of the alkaliphile growing in continuous culture in logarithmic phase under carefully controlled conditions of pH in malate-containing medium

Cytoplasmic pH regulation as the central physiological challenge to extreme alkaliphiles

What basis is there for referring to cytoplasmic pH homeostasis as the central physiological challenge for extreme alkaliphiles? How does such centrality accommodate other important challenges of alkaliphily? How is this centrality different from the importance of pH homeostasis for other bacteria? Most bacteria regulate their cytoplasmic pH, maintaining it in range of pH 7–7.5, but can only grow well up to pH 8.5–9 in media that are highly buffered or otherwise maintained at constant pH during growth (Booth 1985; Padan and Schuldiner 1996). Thus bacteria such as *Escherichia coli* and *Bacillus subtilis* and the marine *Vibrio alginolyticus* evidently have mechanisms that accomplish the acidification of the cytoplasm relative to the external pH at the upper end of their pH range for growth. On the other hand, other prokaryotes and perhaps some archaea may lack such mechanisms, i.e., may simply not have undertaken to meet the challenge of pH homeostasis in an active manner. The results would be anticipated to be a narrower range of pH for growth. *Clostridium fervidus* is proposed to be an example of such a prokaryote that lacks antiporters that are crucial to pH homeostasis in the alkaline range and is restricted to a narrower pH range (Speelmans et al. 1993).

In summary, among neutralophilic organisms, active pH homeostatic mechanisms are found but they are not essential. For alkaliphilic growth, however, they are essential. That they are central is suggested by the finding that with a challenge of increasingly high external pH, the factor that best correlates with the fall-off in the growth rate (i.e., increasing generation time or tg) is the cytoplasmic pH, pH_{in}. This is perhaps best shown in studies of alkaliphilic *Bacillus firmus* OF4 in a pH-controlled chemostat at various values of the medium pH with malate as the growth substrate (Sturr et al. 1994). The cytoplasmic pH, the transmembrane electrical potential ($\Delta\Psi$), and the total Δp were monitored during logarithmic phase growth in the chemostat at specific pH values which allowed calculation of the tg and determination of the molar growth yields on malate. In Fig. 1, data from that study for pH_{in}, pH_{out}, $\Delta\Psi$, and Δp are all plotted against the tg to assess correlation with the tg. First, it is noted that nonfermentative growth of the alkaliphile was excellent at near-neutral pH to pH values above 10.5, as indicated by generation times below 55 min from pH_{out} 7.5 to pH_{out} 10.6, and generation times of no higher than 40 min observed at external pH values between 8.5 and 10.6. There is a definite fall-off in the growth rate that is dramatically observed above pH_{out} 10.6. The correlation of pH_{in} with tg is also clear and this parameter better follows the rise in tg with increasing pH than does the value of the external pH itself. This is suggestive of a close relationship between the eventual, creeping failure of the pH homeostatic mechanisms and the fall-off in growth rate. By contrast, other parameters did not correlate with the tg. The maximal Δp values were observed at slightly submaximal growth rates, and a wide range of growth rates were observed at a single Δp; intermediate values of Δp were observed at the highest tg (i.e., at the highest pH values at which a membrane potential was still found but

the pH homeostatic mechanisms had largely failed). The $\Delta\Psi$ values also correlated poorly with tg, with a similar pattern.

Quite possibly, though, other different alkaliphilic organisms will emerge that have developed ways in which to sustain ever increasing $\Delta\Psi$ values as the external pH is raised; in that event the Δp values and perhaps even the pH_{in} would remain steadier as the external pH rose. It will be of great interest to see if such organisms are discovered and what accompanying membrane lipid properties and primary pump properties they exhibit. It will also be of interest and importance to examine the pattern of one of the alkaliphilic *Bacillus* species in a study of the sort just described, using different growth substrates to determine how the optimal pH and range of pH of growth, as well as other energetic parameters, vary with growth substrate.

Bacillus firmus OF4 and other alkaliphilic *Bacillus* species have been shown to maintain a pH gradient, acid in, of more than two full pH units during optimal logarithmic growth at alkaline pH values. In the experiments shown in Fig. 1, for example, the cytoplasmic pH was over two units more acid than the exterior from medium pH values of 10.6 to 11.2. Other, neutralophilic prokaryotes do not generate a pH gradient, acid in, of this magnitude during growth or when challenged by a sudden alkaline pH shift in the medium (Cheng et al. 1994; Krulwich et al. 1994). Growth on Petri plates or generation time in batch or continuous culture as a function of external pH can be used to assess whether an organism is an alkaliphile. However, a more direct assay of the pH homeostatic property examines the capacity of an organism to establish a relatively acidified cytoplasm during such an alkaline shift (McLaggan et al. 1984). Indeed, there is evidence from experiments with *B. subtilis* that the challenge of a sudden pH shift is a more stringent challenge than growth at elevated pH in a batch culture (Cheng et al. 1996). A caveat for obligate alkaliphiles is that the shift would have to be restricted entirely to a highly alkaline range of pH (e.g., a shift from pH 9.0 to 10.5) because the organism might not retain full functional stability if first equilibrated at near-neutral pH values. For the many useful alkaliphile strains that are facultative, i.e., grow well at both near-neutral and highly alkaline pH values, the pH shift assay is valuable.

The centrality of pH homeostasis as a challenge to alkaliphiles leads to the question of what are the most pH-labile enzymes, structures, and cytoplasmic processes that fail as the cytoplasmic pH rises? No specific information exists on this, although the formation of chains at the upper extreme end of the growth range (Sturr et al. 1994) and the finding of mutations to nonalkaliphiliy with specific morphologies (Hashimoto et al. 1994.) suggest that the cell division machinery might be among them. Similarly, there is no compelling infomation about the possibility that specific classes of cytoplasmic macromolecules might be protected by particular passive mechanisms against the possibility of occasional adverse rises in cytoplasmic pH. Nor does the proposal that pH homeostasis is a central challenge detract from other necessary and/or intriguing adaptations that alkaliphiles must have made. Most obvious is the necessity that all structures and enzymatically active proteins or parts of proteins that are exposed to the highly alkaline exterior must be able to retain functional stability in this milieu. In fact, a powerful argument against any suggestion that the pH just outside the cytoplasmic membrane of alkaliphiles is not unusually alkaline (being protected, perhaps, by special wall layers) is the finding that the external loops of alkaliphile polytopic membrane proteins show a marked and characteristic reduction in basic and increase in acidic amino acid residues relative to neutralophile homologues of the same proteins (Kang et al. 1992; Hicks and Krulwich 1995; Ito et al. 1997b). If, as assumed, this is an important adaptation, then mutants in any physiologically important polytopic membrane protein, exoenzyme, or extracellular structure that significantly altered this pattern would be expected to result in a nonalkaliphilic phenotype, even if the molecule or process was not specifically key to alkaliphily per se. Certainly, nonalkaliphily would result if the catalyst were part of the pH homeostatic mechanism.

Additional challenges to the alkaliphile emerge from the successful resolution of the pH homeostasis problem. As is evident in the data shown in Fig. 1, the total chemiosmotic driving force that is available for bioenergetic work is highest at near-neutral pH values rather than at the highest and most optimal pH values for growth. This raises questions about the details of the oxidative phosphorylation mechanism of extreme alkaliphiles (Guffanti and Krulwich 1994; Krulwich 1995). However, while this would be a major problem for an alkaliphilic *Bacillus* growing on a nonfermentative carbon source, it might be completely irrelevant when growth is on glucose and a rich medium, whereas pH homeostasis is still an important function under these conditions (Gilmour and Krulwich 1997).

There follows a review of the specific mechanisms that have been advanced or explored as having a role in alkaliphilic pH homeostasis. Embedded in the discussion will be attempts to outline a possible analysis of the limitations of each mechanism. Perhaps there will ultimately be opportunities, by natural selection or by experimental design to develop "hyperalkaliphilic" strains. Such strains might arise from enhanced function at the limits of the homeostatic mechanism and might also result from changes that reduce the lability of the most alkali-labile sites of cellular physiology.

Passive defenses against cytoplasmic alkalinization

The striking primary sequence deviation of alkaliphile proteins that are immediately outside the cytoplasmic membrane from neutralophilic homologues is inconsistent with a mechanism that posits complete protection of the region near the coupling membrane from the alkaline external pH. However, no direct measurement of the pH near or at the external surface has yet been made. It is possible that some partial protection occurs or that upon an alkaline shift there is at least transient protection offered by structures outside

the cytoplasmic membrane. Aono, Horikoshi and their colleagues have noted that the facultatively alkaliphilic *Bacillus* C-125, which is related to *Bacillus lentus* (Aono 1995), contains substantial amounts of two acidic polymers, a teichuronic acid and a glutamate-rich teichuronopeptide whose mutational loss leads to poor growth on glucose at alkaline pH (Aono and Ohtani 1990; Aono et al. 1995). A similar teichuronopeptide has been found in the cell walls of another group of alkaliphilic *Bacillus* sp. (Aono et al. 1993) but has not been found in all alkaliphilic *Bacillus* sp. (Guffanti and Krulwich 1994). It is hypothesized that a high cell wall negative charge is necessary for at least some groups of alkaliphilic bacteria, where it may provide a hindrance to entrance of hydroxide ions (Aono and Ohtani 1990). Alternatively, the carboxylates might bind divalent metal cations, resulting in a net positive charge that limits proton loss from the space in between the membrane and the wall in a manner similar to that proposed by others (Kemper et al. 1993). Whether these compounds are produced in a pH-dependent manner or abundantly at all pH values during growth of the alkaliphilic *Bacillus* species such as *Bacillus* C-125 on nonfermentative carbon sources is yet to be determined. Conversely, the possibility exists that alkaliphilic strains that have not been found to have particularly elevated amounts of such a polymer relative to *B. subtilis*, in experiments conducted on malate-grown cells (Guffanti and Krulwich 1994), might yet be shown to have teichuronic acids or teichuronopeptides of physiological importance when grown on glucose. Importantly, the acidic cell wall polymers of glucose-grown *Bacillus* C-125 may be necessary but are clearly not sufficient for pH homeostasis. As will be detailed later, the need for active mechanisms is apparent for this strain (Hamamoto et al. 1994).

Indeed, most totally passive mechanisms would become saturated and lose effectiveness in the face of an ongoing challenge or sudden shift to alkali of sufficient magnitude. Thus, it was reasoned that the high cytoplasmic buffering capacity observed in two malate-grown alkaliphilic *Bacillus* species at very alkaline pH values might reflect specific, undetermined basic compounds that protected particular alkali-labile macromolecules, rather than a defense against global cytoplasmic alkalinization (Krulwich et al. 1985a). Possibly, however, a high buffering capacity of the cytoplasm might offer partial and transient global protection against a sudden pH shift even though active mechanisms are definitely essential. Another consideration with respect to fermentative vs nonfermentative growth of alkaliphiles is whether metabolic acids produced during growth on the former may assist in acidifying the cytoplasm. Some greater contribution of Na^+-independent mechanisms, presumed but not directly shown to be passive types of mechanisms, to cytoplasmic pH regulation has recently been demonstrated for glucose-grown vs malate-grown cells of *B. firmus* OF4 (Gilmour and Krulwich 1997). As shown in Table 1, malate-grown cells that were equilibrated at pH 8.5 in malate-containing buffer and then shifted to pH 10.5 in the presence of malate, were totally dependent upon Na^+ for pH homeostasis; in the absence of Na^+ the cytoplasmic pH was the same as the new external pH within 10 min whereas when Na^+ was present homeostasis was achieved. Glucose-grown cells of either wild type or a mutant that was disrupted in the operon encoding the caa_3-type terminal oxidase of the respiratory chain exhibited almost the same capacity for pH homeostasis as malate-grown cells, with a small consistent defect evident in the mutant strain. However, the glucose-grown cells could maintain a partial pH gradient, acid in, up to 10 min after the shift even in the absence of Na^+.

Table 1. The Na^+-dependence of pH homeostasis in glucose-grown vs malate-grown cells of *Bacillus firmus* OF4

Growth and energization substrate	Strain[a]	pH_{in}, 10 min after pH 8.5→10.5 shift, in	
		Na_2CO_3	K_2CO_3
Glucose	Wild type	8.39 ± 0.15	9.17 ± 0.09
Glucose	D-11 (CtaD::spc^R)	8.67 ± 0.08	9.29 ± 0.09
Malate	Wild type	8.38 ± 0.11	10.48 ± 0.05

Cells growing at pH 10.5 on glucose or malate were washed and resuspended at pH 8.5 as described elsewhere (Ito et al. 1997a) and subjected to a pH shift in the external medium to 10.5; after 10 min the cytoplasmic pH was measured (Gilmour and Krulwich 1997). Four determinations were made for glucose-grown cells and eight for malate-grown cells.

[a] The wild type strain used was the 811M strain of *B. firmus* OF4 and the D-11 strain is disrupted in the *cta* operon as described (Gilmour and Krulwich 1997).

Active mechanisms for cytoplasmic pH regulation in alkaliphiles

Work in several different laboratories has contributed to a consensus that growth of extremely alkaliphilic *Bacillus* species at the upper end of their pH range is dependent upon the presence of Na^+ even though these nonmarine, nonhalophilic organisms may require only low concentrations of Na^+ that are sometimes difficult to discern even in defined media. The requirement for Na^+ relates to both pH homeostasis and the uptake of many solutes, with Na^+/solute symport being both intrinsically important in alkaliphile physiology as well as a component of the pH homeostatic cycle. That cycle is envisioned as containing the elements outlined schematically in Fig. 2. It is initiated by the primary extrusion of protons, by the electron transport chain in respiring cells, and probably at least in part by the H^+-coupled F_1F_0-ATPase in cells that are growing fermentatively. The Δp thus established energizes electrogenic antiporters that catalyze net uptake of positive charge during the exchange of cytoplasmic Na^+ ions for a greater number of protons in each cycle; the precise stoichiometry is not yet known for any of the alkaliphile antiporters. Net proton accumulation can be achieved by the combined functions of the primary pump(s) and secondary, electrogenic antiporters. As already noted, this cycle is Na^+-specific, as far as is currently known, in alkaliphiles growing on nonfermentative carbon sources, whereas neutralophiles, e.g., *B. subtilis*, can use K^+/H^+ antiporters or

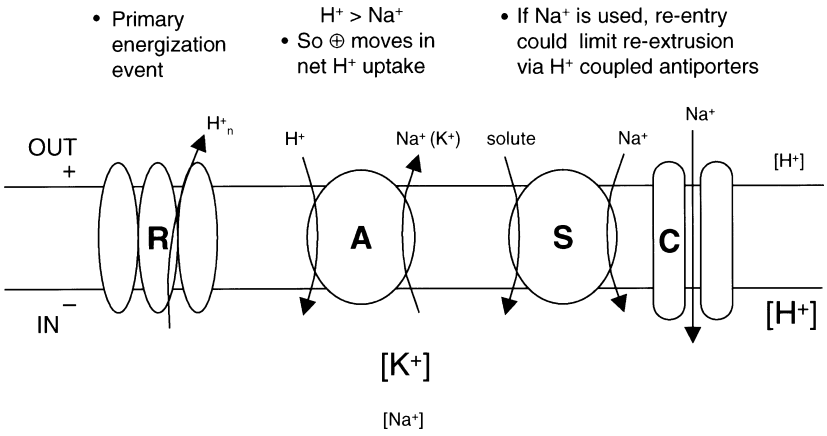

Fig. 2. A schematic representation of the membrane-associated elements associated with the putative active cycle for pH homeostasis that, in an exclusively Na^+-coupled mode, is crucial for alkaliphily. *R*, respira-tory chain; *A*, monovalent cation/H^+ antiporter(s); *S*, Na^+/solute symporters; *C*, Na^+ channel, e.g., the one associated with alkaliphile motility

antiporters that use either Na^+ or K^+ (Krulwich et al. 1994; Cheng et al. 1994, 1996). Thus, the alkaliphiles must also possess mechanisms for rapid recycling of the Na^+ to make cytoplasmic cation available when sustained antiporter activity is required, i.e., during an alkaline shift and throughout periods of growth at extremely alkaline pH. There is evidence that the numerous Na^+/solute symporters play a significant role in this Na^+ re-entry (Krulwich et al. 1985b) and it has been hypothesized that there are pH-gated Na^+ channels to provide an additional crucial component to this part of the cycle, especially when solute concentrations are low (McLaggan et al. 1984). The possibility that the Na^+-translocating channel associated with alkaliphile motility could fulfill this function has been outlined by Sugiyama (1995).

It is not established that this simple cycle is sufficient to achieve and sustain a pH gradient, acid in, that is over 2 full pH units without completely dissipating the $\Delta\Psi$ that must be retained for other bioenergetic work. The problem is especially cogent during growth on nonfermentative substrates, where that other bioenergetic work is substantial and the participation of passive mechanisms has not been clearly shown. The cycle may not be fully sufficient in the absence of passive mechanisms and, perhaps, there may be additional active components that are yet to be identified. However, the Na^+-dependent active cycle is clearly necessary under all highly alkaline growth conditions. Mutations in the Na^+/H^+ antiporter activity of alkaliphile cells and membranes result in a nonalkaliphilic strain (Kitada et al. 1989; Garcia et al. 1983; Hamamoto et al. 1994). In addition, there may be special permeability properties of the coupling membrane or special mechanisms associated with this cycle itself that are connected to the alkaliphile's remarkable efficacy in pH homeostasis relative to that of neutralophiles. For example, there might be membrane-associated binding proteins or other devices that produce a higher Na^+ concentration near the antiporters, at the cytoplasmic side of the coupling membrane, and thus enhance antiport activity. A localized increase in Ca^{2+} concentration has been posited in connection with specific neural membranes (Tank et al. 1988).

As expected, the function of the respiratory chain has an impact on pH homeostasis, even a modest one during growth on nonfermentative carbon sources (Table 1). Respiratory chain components and the rates of oxygen consumption via respiration are elevated variously in different alkaliphiles during growth at high vs near-neutral pH, consistent with the importance of the primary energy generators in alkaliphily in general (Hicks and Krulwich 1995; Krulwich et al. 1996; Aono et al. 1996; Qureshi et al. 1995); pH homeostasis is certainly one of the energy costs at high pH that place stringent demands upon the overall cellular energetics.

What is known about the antiporters that are specifically involved in pH homeostasis? Thus far, it is evident that alkaliphile cells possess multiple Na^+/H^+ antiporters (Kitada et al. 1994; Ito et al. 1997a) and that at least three distinct antiporters are likely to function in a single alkaliphilic *Bacillus* such as *B. firmus* OF4 (Ito et al. 1997a). In that organism, there are apparently at least two high affinity Na^+/H^+ antiporters, one of which is constitutive and one of which is induced at high pH. The constitutive one is the product of the *nhaC* gene, that was identified via its functional complementation of a Na^+/H^+ antiporter-deficient *E. coli* strain (Ivey et al. 1991). Targeted disruption of *nhaC* in *B. firmus* OF4 results in a diminished capacity for Na^+-dependent adjustment to a pH shift at very low Na^+ concentrations, and an impaired cycling of Na^+ at near-neutral pH (Ito et al. 1997a).

B. firmus OF4 also possesses at least one major and largely constitutive Na^+/H^+ antiporter that is likely to be a moderate to low affinity high V_{max} antiporter; a mutation in this antiporter would be expected to result in significantly diminished ability to grow at high pH. It is possible that this antiporter will be a homologue of an important antiporter identified in *Bacillus* C-125 (Kudo et al. 1990; Hamamoto et al. 1994). In those studies, a nonalkaliphilic mutant of the alkaliphile with deficient Na^+/H^+ antiport activity was complemented by a homologous DNA library. The gene focused upon in the report was the first open reading frame (ORF) of an apparent operon; it was predicted to encode a polytopic membrane protein with modest similarity to

NADH dehydrogenases, especially in the N-terminal part, and to NhaC, especially in the C-terminal part. Complementation occurred by recombinational correction of a point mutation in between these regions (Hamamoto et al. 1994) and complementation in the absence of such a crossover was not demonstrated. Indeed, this gene is probably part of a large locus encoding multiple hydrophobic proteins since close homologues are in the databases from *B. subtilis* (accession number Z93937) and *Rhizobium meliloti* (accession number X93358). It will be of great interest to determine the roles of the various gene products and, in particular, the differences that may exist between homologous alkaliphile and neutralophile antiporters, e.g., that may explain the apparent Na^+ specificity of alkaliphile pH homeostasis. It is hypothesized that the alkaliphile may utilize a completely or relatively Na^+-specific process in order to preserve cytoplasmic K^+ under conditions of extreme pH stress (Ito et al. 1997b).

The total number of different antiporters used for pH homeostasis at different Na^+ concentrations and, perhaps, in relation to other variables, is yet to be determined rigorously for any single alkaliphilic *Bacillus*; this would be done by identification of all the genes and their mutational disruption singly and in combinations. It will be of interest to see if indeed they are all Na^+/H^+ antiporters or whether K^+/H^+ or $Na^+(K^+)/NH_4^+$ antiports may play some role.

When the molecular participants in the active cycle on which pH homeostasis depends are more fully characterized, it will be of further interest to explore what fails when the capacity is pushed to the limits by elevation of the growth pH. There is some evidence that growth of an alkaliphile in the chemostat at pH values over 11 results in the emergence of strains with elevated membrane antiport activity (Sturr et al. 1994) but the antiporter involved and the sufficiency of that change have not been examined. It is quite likely that optimal production of "hyperalkaliphiles" could require changes in several different genes, possibly including those affecting membrane lipids, respiratory chain or other primary Δp generators, antiporter(s), and Na^+ re-entry routes.

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