The Chromosomal Tetracycline Resistance Locus of Bacillus subtilis Encodes a Na⁺/H⁺ Antiporter That Is Physiologically Important at Elevated pH*

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The chromosomal tetB(L) gene of Bacillus subtilis encodes a transporter that catalyzes Na⁺/H⁺ antiport even more actively than tetracycline/H⁺ antiport, as shown by assays of membrane antiporter activity upon transformation of Na⁺/H⁺ antiporter-deficient Escherichia coli with the cloned gene; the transformation results in a substantial increase in Na⁺ resistance as well as detectable resistance to low tetracycline concentrations. Transpositional disruption of the chromosomal tetB(L) locus of B. subtilis led to reduced rates of electrogenic Na⁺ efflux and revealed a physiological role for this locus in Na⁺ resistance and Na⁺-dependent pH homeostasis at pH 8.5. The mutant phenotype was reversed by transformation with a plasmid expressing the cloned tetB(L) gene. Energy-dependent tetracycline efflux rates of the wild type were greater than in the transposition mutant but were not sufficient to confer resistance to the antibiotic. TetB(L) is also inferred to have a modest capacity for K⁺ efflux, since the transposition mutant is slightly impaired in K⁺-dependent pH homeostasis at pH 8.5 and grew better than the wild type at pH 7 on limiting K⁺ concentrations.

The clinical efficacy of tetracycline as an antibacterial agent has been severely compromised during the past several decades by the proliferation of resistant organisms that carry several distinct categories of determinants (1–3). A major subset of these determinants encodes efflux proteins that limit the accumulation of the drug in the cytoplasm of otherwise sensitive bacteria (2). For this and other efflux proteins that confer antibiotic or multidrug resistance, there is presumably a physiological role that accounts for their presence, or they evolved from proteins that had such a role in the organism(s) of origin (1, 4). There are roles for proteins that catalyze drug efflux in the organisms that produce the drug or live in the proximity of producer strains (5, 6), but even those proteins may have evolved from efflux systems that have substrates of more general physiological importance. Recent evidence indicates, for example, that one of the eukaryotic multidrug resistance efflux systems plays a physiological role in drug elimination by the brain (7). Insights into the underlying or original physiological roles can be helpful in understanding the factors that contribute to the development of resistance to any particular agent and perhaps can also contribute to strategies aimed at circumventing or preventing the resistance. Since tetracycline is still in use in both human and veterinary biomedicine, such insights could have specific as well as extrapolative value. Accordingly, it was intriguing to discover that disruption of the chromosomal tet locus of Bacillus subtilis resulted in a phenotype that we were using to identify genes encoding Na⁺/H⁺ antiporters.

Tetracycline resistance determinants that function via protein-catalyzed efflux of the antibiotic have long been recognized in numerous plasmids and transposons from both Gram-negative (8) and Gram-positive bacteria (9, 10). In addition, an efflux type of tet gene exists in single copy near the origin of replication of some B. subtilis strains that are nonetheless tetracycline-sensitive (11). This gene, designated terB, was first recognized subsequent to the detection of tetracycline resistance after mutagenesis (12). The terB gene, or possibly a distinct gene that was also near the origin of replication, was later shown to confer tetracycline resistance upon amplification, after wild type cells were subjected to certain protoplast regeneration protocols (13, 14), or upon expression of the gene on multicopy plasmids (15, 16). The chromosomal tetB gene, which will be referred to as tetB(L), according to the nomenclature proposed by Levy et al. (17), has remained an unexplained, presumably cryptic oddity (2). However, it has been noted that elucidation of a physiological role for this locus might clarify the origins and properties of this class of resistance determinants (1, 2). The current study indicates a set of physiological functions for this locus that relate to a heretofore unrecognized capacity for monovalent cationic proton exchange by TetB(L). A preliminary report of some of the findings was presented at a meeting on Porters in June, 1994 (18).

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Growth Conditions—B. subtilis BD99 (obtained from Dr. A. Garro) was used as the wild type. The transpositional libraries were those that had been prepared in the course of earlier studies (19) using plasmid pLT1, which contained a derivative of Tn917 (20). Plasmid pCIS7, containing the tetB(L) locus (16), was obtained from Dr. K. Bott. JC111 was made competent and transformed with pCIS7 according to the procedure of Dubnau and Davidoff-Abelson (21); transformants were selected by their resistance from the transpositional libraries was carried out on plating media of the same composition as the routine growth medium, at pH 7 (non-selective), and colonies were replicated onto media at pH 7 containing 0.7 M NaCl, or at pH 9 containing 0.3 M NaCl. For subsequent growth studies, liquid media were prepared at either pH 7 or 8.5 containing: 100 mM Tris-HCl buffer, 1 mM potassium phosphate, 0.01% MgSO₄, 0.2% NH₄SO₄, 50 μg/ml each of threonine, histidine, and tryptophan, and 0.1% yeast extract. For studies of Na⁺ resistance, 50 mM potassium
B. subtilis tetB Encodes a Na⁺/H⁺ Antiporter

Malate was used as the carbon source, and NaCl was added as indicated. For studies of alkali sensitivity as a function of the monovalent cation composition of the medium. 50 mM Tris malate was used as the carbon source, and additions of either KCl or NaCl were made as indicated. This same basal medium, at pH 7, was used for studies of growth inhibition by tetracycline. Growth studies were conducted by incubating 2 ml of cultures in 15-ml tubes, with shaking at 30 °C, for 16 h, after which the OD₆₀₀ was determined. All growth experiments were conducted two to four times using duplicate to quadruplicate samples. Escherichia coli DH5αMCR (Life Technologies, Inc.) was used in determination of the sequence of the insertional site and for cloning of the tetB gene. E. coli NM81, a strain in which the Na⁺/H⁺-encoding nhaA gene is disrupted (23), was obtained from Dr. Etana Padan, and used for the functional complementation studies and antiporter assays. The bacteria were grown in Luria broth (LB) at 37 °C, with 100 µg/ml of ampicillin added when appropriate; for E. coli NM81, the NaCl in LB was replaced with KCl (LBK medium), and kanamycin was added (50 µg/ml).

Analyses of the Insertional Site in JC111—The region flanking the insertional site in JC111 was cloned using the strategy developed by Camilli et al. (20), precisely as described in connection with an earlier use of the same transpositional libraries (19). Digestion of chromosomal DNA from JC111 was carried out with EcoRI and the flanking region of the transposon together with partial sequence of Tn917 was self-ligated to create the new plasmid pHPA111. The nucleotide sequence of the insert in pHPA111 was determined using an Applied Biosystems 373A automated sequencer in the DNA Core Laboratory of the Mount Sinai School of Medicine. Routine molecular biological procedures were carried out as described by others (24). Southern analyses were conducted using probes prepared by polymerase chain reaction and corresponding to 785 base pairs of the internal sequence of the tetB gene and 430 base pairs of the cat gene of the Tn917 (19).

Determination of Cytoplasmic pH upon a pH Shift in the Medium—Cells growing log-linearly at pH 7 on Tris-Tris malate medium were harvested by centrifugation and resuspended to 1 mg protein/ml in 100 mM Tris buffer, pH 7.5, plus 10 mM Tris malate. The cells were rapidly diluted 25-fold by the addition of 200 µl of the cell suspension into 5 ml of one of the following three buffers at pH 8.5: 100 mM Tris, 100 mM potassium phosphate, or 100 mM sodium phosphate. The pH of the buffers was monitored after the introduction of the cells, and the APH across the membranes was determined, as described elsewhere (25), by the distribution of a radiolabeled weak base or acid using a filtration assay (26). Samples were taken 10 min after the pH shift. Controls for nonspecific binding of the probes consisted of cells to which 10 µM carbonyl cyanide m-chlorophenylhydrazone, 10 µM gramicidin, or 5% butanol was added. The background binding of the basic probe was particularly high in the Tris-containing buffer but did not interfere with measurement of pH gradients in additional control experiments in which potassium or sodium salts were also present. Values for cytoplasmic pH were determined from duplicate measurements of the external pH and the APH, in six independent experiments.

Efflux of [32P]Tetracycline from Whole Cells—Cells were partially depleted of energy sources and loaded with either [32P]Tetra or [3H]Tetracycline. For experiments in which [32P]Tetra efflux was driven by respiration, the cells were grown in Tris-Tris malate medium at pH 7, washed, and suspended in 100 mM Tris buffer, pH 8.5, to 20 mg protein/ml. When efflux was driven by a potassium diffusion potential the cells were washed and suspended to the same concentration in 100 mM potassium phosphate buffer, pH 8.5, in the presence of 1 mM valinomycin. The cells were passively loaded by incubation with 5 µM [32P]Tetra at 21 °C for 15 min. Efflux driven by respiration was initiated by diluting 2 µl of cells at 21 °C into 400 µl of 100 mM Tris buffer, pH 8.5, containing 10 mM Tris malate. In the efflux potential-driven efflux experiments, 2 µl of cells were diluted into 400 µl of either 100 mM Tris buffer, pH 8.5, or 5.5 mM K₂HPO₄, pH 8.5, both of which contained 1 µM valinomycin. For tetracycline efflux, the cells were washed and suspended in 100 mM Tris buffer, pH 7, without or with either 100 mM NaCl or 100 mM KCl. The cells were loaded with solute by incubation for 1 h at 21 °C in the presence of 1 µg/ml of [3H]Tetracycline. Efflux of tetracycline was initiated at 21 °C by the dilution of 2 µl of cells into 400 µl of 100 mM Tris buffer, pH 7, plus 10 mM Tris malate in the presence of the same amount of added cation that had been present in the loading buffer for a particular sample. Samples were taken, filtered, and counted as described previously (27).

Cloning of the Chromosomal tetB(L) Gene—The tetB(L) gene was amplified by polymerase chain reaction from chromosomal DNA from the wild-type strain of B. subtilis used in this study using the primers GGAGGGGGATCCATTAGATACCGTCCTATTCAAG and TTTTCA-

**Fig. 1. Inhibition of growth of wild type, JC111, and JC111/pcIS7 by added NaCl in potassium replete medium at pH 8.5.** Cells were grown on medium buffered with Tris at pH 7 or 8.5 and containing 50 mM potassium malate as carbon and potassium sources. The indicated concentrations of NaCl were added to cultures at pH 8.5. The OD₆₀₀ was measured after 16 h of growth.

AGCTTAGCCATGTCCCTCAGCAAG. The amplified product was gel purified and blunt end ligated into the HincII site of pGEM3Zff(-). A plasmid designated pJTAl, which had tetB(L) inserted under control of the T7 promoter, was transformed into E. coli NM81; other heterologous antiporter genes had functionally complemented E. coli NM81 in this manner (28).

**Assays of Antiporter Activities in Everted Membrane Vesicles—**Everted membrane vesicles were prepared from E. coli NM81 transformed with either pJTAl or pGEM3Zff(-), by the method of Ambudkar et al. (29) as described previously (28), except that in some experiments the growth medium (LBK) for the transformant with pJTAl contained 0.5 mM NaCl. The antiporter activities were assayed by a fluorescence assay in which the quenching of acridine orange is monitored as described earlier, in buffers at pH 8.0, containing 5 mM MgCl₂ (28, 30).

**RESULTS**

**Disruption of the tetB(L) Locus of B. subtilis—**Transposition libraries of B. subtilis containing Tn917 insertions were screened for Na⁺- and/or alkali-sensitive mutants by replica plating from malate-containing Spizizen's medium at pH 7, with no added NaCl, to plates at either pH 7 or 9, containing 0.7 and 0.3 mM NaCl, respectively. Strains of interest were found only on the high pH-NaCl plates. Each of five strains was confirmed, by growth experiments in liquid culture, to be Na⁺ sensitive relative to the wild type. Upon sequencing of the DNA flanking the transposon in strain JC111, the insertion was found to be in the promoter region of the tetB(L) gene, in between the two base pairs that are in positions −27 and −26 relative to the putative transcription start site proposed by Sakaguchi and Shishido (15). The results of Southern analyses of EcoRI and HpaI digests of chromosomal DNA from wild type and JC111 were consistent with the sequence data on the flanking region of the insertion and showed that there was only a single Tn917 insertion in JC111.

Growth of JC111 was compared to the wild type and to JC111 transformed with pCIS7, a plasmid carrying the tetB(L) gene as cloned by Ives and Bott (16, 31). A strongly buffered liquid medium was prepared using Tris buffers at either pH 7 or 8.5. For assessments of Na⁺ sensitivity, potassium malate was used as the carbon source, thus making the potassium concentration of the basal medium 100 mM. NaCl was added to final concentrations of 50, 100, or 200 mM NaCl. As shown in Fig. 1, wild
indicated that mixtures of the Tris and phosphate buffers yielded the buffer, at pH 8.5, composed of Tris, sodium phosphate, or potassium in the same patterns as the phosphate buffers alone.

Wild type and JC111, with the efflux from JC111 consistently and significantly slower than that from the wild type in the absence of uncoupler (data not shown). In the second protocol, energization was achieved by imposition of a valinomycin-mediated potassium diffusion potential which would energize an electrogenic antiport that could respond to the potential formed; the control in this protocol involved dilution of the NaCl- and potassium-loaded, valinomycin-treated cells into medium in which a high concentration of potassium prevented the development of a potential. As shown in Fig. 3, rapid diffusion potential-dependent efflux of NaCl was observed in both strains, but efflux from the wild type was much more rapid than in JC111. The potential-independent component was the same in the two strains.

Tetracycline Eflux and Growth Inhibition by Tetracycline—It was of interest to determine whether the disruption in the chromosomal tetB(L) locus could be detected via a tetracycline efflux or sensitivity, even though this locus has been considered entirely cryptic in single copy. Cells of wild type B. subtilis and JC111 were loaded with [3H]tetracycline at pH 7, in the absence of added monovalent cations or in the presence of either 100 mM KC1 or 100 mM NaCl. At zero time, the cell suspensions were diluted into Tris buffer at pH 7, containing 10 mM Tris malate in the absence or presence of 100 mM KC1 or NaCl. As shown in Fig. 4, efflux of tetracycline was minimal in JC111 under any of the conditions, whereas efflux from the wild type was evident over the early time points. Loading of the wild type in buffer containing sodium resulted in a significantly lower level of [3H]tetracycline at the earliest point taken after dilution. This might indicate a Na+-dependent component of the accumulation of tetracycline during the preloading (data not shown).

Since there was a distinct decrease in tetracycline efflux upon disruption of tetB in JC111, it was anticipated that a slightly greater sensitivity of the mutant strain to growth inhibition by low concentrations of the antibiotic might also be observable. When examined in Tris-buffered medium, at pH 7,
B. subtilis tetB Encodes a Na+/H+ Antiporter

27368

containing Tris malate and only minimal (1 mM) potassium, the opposite result was surprisingly found. As shown in Fig. 5, JC111 grew better than the wild type at low concentrations of tetracycline. If the tetracycline up-regulated the cellular level of the TetB(L) protein, and TetB(L) could use potassium as a substrate in addition to sodium and tetracycline, then potassium depletion might be a major inhibitory factor under these conditions. Addition of a higher concentration of potassium, but not of sodium, indeed eliminated the greater inhibition of the wild type at low tetracycline concentrations (Fig. 5). However, even in the presence of sufficient potassium to eliminate the growth inhibitory effect of a functional tetB(L) gene, no tetracycline resistance was observed in the wild type as compared to JC111.

Expression of the Cloned tetB(L) from Wild Type B. subtilis in an Antiporter-deficient E. coli Strain—The experiments with JC111 suggested that the chromosomal tetB(L) gene encodes an antiporter that has physiologically significant Na+/H+ antiport activity, as well as measurable tetracycline/H+ antiporter activity, although the latter is insufficient to confer antibiotic resistance. To test this directly, the tetB(L) transporter gene was amplified alone from B. subtilis chromosomal DNA by polymerase chain reaction and cloned into pGEM3Zf(+) under the T7 promoter. The resulting plasmid pJTAl was transformed into E. coli strain NM81. Growth of this strain is inhibited by NaCl concentrations above 0.4 M at pH 7.5 because of a deletion in the antiporter-encoding nhaA gene (23); growth of E. coli NM81 is also completely inhibited by tetracycline concentrations as low as 2 μg/ml. Upon transformation by pJTAl, the strain exhibited growth on plates of LBK medium containing up to 0.6 M NaCl or up to 4 μg of tetracycline/ml, which did not allow growth of the same strain transformed with pGEM3Zf(+). Growth of the pJTAl transformant in liquid LBK medium led to loss of the transformed phenotype.

Everted membrane preparations from the control and pJTAl transformants of E. coli strain NM81 were assayed for antiporter activities. In the fluorescence assays used, and depicted in Fig. 6, the quenching of fluorescence upon addition of the electron donor, D-lactate, reflects the respiration-dependent establishment of a pH gradient, acid inside the everted vesicles. The antiport activity with a particular substrate, i.e. Na+ or tetracycline, is then measured semiquantitatively by dequenching, reflecting proton efflux from the vesicle that occurs upon addition of the other antiport substrate to the outside. As shown in Fig. 6, control vesicles of E. coli strain NM81 transformed with pGEM3Zf(+) had low levels of antiport activity for both Na+ and
B. subtilis tetB Encodes a Na+/H+ Antiporter

27369

tetracycline, the activity with tetracycline being slightly greater. The transformant with pJTAl, containing the cloned tetB(L) gene, generated a smaller pH gradient than the control transformant, suggesting that the TetB(L) product compromises membrane function in the heterologous host; this is presumably the basis for the selective pressure against the multicopy recombinant plasmid. However, the Na'/H+ antiport activity of the E. coli/pJTAl transformant was consistently and markedly higher than that of the control. The activity with tetracycline was less impressive but in several determinations was significantly higher than the control when calculated as the % dequenching. That the cloned tetB(L) gene does in fact enhance tetracycline uptake by the everted vesicles was confirmed with experiments using [3H]tetracycline to monitor transport (data not shown). The activity of K'/H+ antiport was even closer to the low level of the control and cannot be assessed as positive on the basis of this assay (data not shown).

DISCUSSION

The chromosomal tetB(L) gene of B. subtilis encodes a tetracycline efflux antiporter, as expected from sequence data and prior reports (15); it is active in cells albeit insufficient to confer resistance. The current results show that the same gene product is an even more active electrogenic Na'/H+ antiporter that plays a physiological role in Na+ resistance and in Na+-dependent pH homeostasis at alkaline pH values. The concomitant increase in Na+ resistance, Na'/H+ antiport, tetracycline resistance, and tetracycline/H+ antiport upon expression of the cloned tetB(L) gene in E. coli establishes the gene product as an efflux protein that uses Na+ in addition to tetracycline. This is the first demonstration of the activity of Tet proteins in Na+ efflux and of physiological roles for tet gene products that are unrelated to tetracycline. TetB(L) probably functions as a Na'/H+ antiporter at neutral pH as well as at pH 8.5. At neutral pH, it would lower the proportion of the electrochemical proton gradient that is in the form of a ΔpH, thus accounting for the reduced loading of wild type versus JC111 cells with tetracycline when Na+ was present (Fig. 4). Since the cloned tetB gene complemented the phenotype of JC111, the single transpositional event rather than some secondary mutation was the basis for that phenotype.

The diminution in diffusion potential-dependent Na+ efflux as well as uncoupler-sensitive Na+ efflux indicates that Na'/H+ antiport by TetB(L) is electrogenic, i.e. proceeds with a H+/Na+ ratio greater than one. Na'/H+ antiporters are ubiquitous transport proteins that have been implicated in numerous physiological processes, including Na+ resistance and pH ho-

Fig. 5. The inhibition of growth of wild type and JC111 by tetracycline at pH 7 in the absence and presence of 100 mM KCl or NaCl. The cells were grown in the low potassium medium described in the legend to Fig. 3, except that here the pH was 7 for all samples. The indicated additions of KCl, NaCl, and tetracycline were made. The OD600 was recorded after 16 h of growth.

Fig. 6. Na+/H+ and tetracycline/H+ antiport activity of everted membranes of E. coli NM81 transformed by pJTAl, carrying tetB(L), or a pGEM3Zf(+) control. Everted vesicles from E. coli NM81, transformed with the indicated plasmid, were energized in the presence of the ΔpH probe acridine orange by the addition of lactate (second arrow in each tracing). At the third arrow, pointing upward at the bottom of the trace, either 10 mM NaCl or 5 μg tetracycline/ml was added. In the traces shown, equal amounts of membrane protein (30 μg/ml of assay mix) were used for the different preparations and assays. Additional experiments were conducted in which the concentration of protein used from the control preparation was chosen so that the initial lactate-dependent acidification of the vesicle interior, i.e. the magnitude of the initial quenching was the same as in the preparations from the transformants with pJTAl; the % dequenching observed with both substrates was unchanged in the range of membrane protein used.
meostasis, and also encompassing osmoadaptation and signaling (33–36). In extreme alkaliophiles, it is clear that Na+/H+ antiporters play a crucial role in pH homeostasis (33, 37). A comparable function has been inferred for the Na+/H+ antiporter gene sod2 in the neutrophilic yeast Schizosaccharomyces pombe (38). The specific importance of electroneutral rather than electroneutral antiport for pH homeostasis has been well described (39). Nonetheless, in E. coli, in which the Na+/H+ antiporters have been most extensively characterized, the nhaA and nhaB genes that encode major, electronegative Na+/H+ antiporters have been shown to function in Na+ resistance but have not yet been shown to be important for growth at high pH or pH homeostasis per se (36, 40). The current study suggests that in contrast to alkaliphilic prokaryotes, in which pH homeostasis specifically requires Na+ (41), neutralophilic prokaryotes can use K+/H+ antiport as an alternative way of catalyzing acidification of the cytoplasm relative to the external medium, as has been previously suggested (42). In B. subtilis, either Na+ or K+ can evidently support both growth at pH 8.5 and pH homeostasis upon a sudden upward shift in the external pH. Sodium appears to be slightly more efficacious than potassium in the shift experiment, which is probably an assay that is more specifically and stringently targeted toward pH homeostasis than is growth at pH 8.5. The disruption of tetB(L) caused a total loss of the capacity to use sodium to regulate cytoplasmic pH during a sudden shift in the external pH, although JC111 retained 50% or more of the wild type capacity for Na+-dependent growth at pH 8.5. JC111 also retained some capacity to grow at elevated pH in K+-replete medium containing 100 mM added NaCl, i.e. retained appreciable Na+ resistance. These results indicate that TetB(L) is the major but not the exclusive Na+/H+ antiporter supporting Na+ resistance and pH homeostasis.

JC111 also exhibited a small, but consistent loss in the K+-dependent pH homeostasis and growth at pH 8.5. This suggested that in addition to catalyzing Na+/H+ antiport, TetB(L) catalyzes K+/H+ antiport, although it has not yet been possible to convincingly demonstrate this upon transformation of E. coli NM81 by pJT1A1; further attempts will be made to optimize the assay for this substrate. In any event, there must be one or more other K+/H+ antiporters that account for the majority of K+-dependent pH homeostasis. A small capacity of the TetB(L) for K+/H+ antiport probably provides the explanation for the otherwise puzzling findings with respect to tetracycline resistance and efflux in the presence and absence of a functional chromosomal tetB(L) gene. JC111 exhibited almost no efflux of tetracycline, whereas the wild type strain exhibited significant initial efflux. Nonetheless, when examined in medium containing a growth-limiting concentration of potassium, tetracycline was more inhibitory to the wild type. Since addition of higher potassium concentrations, but not sodium, alleviated this difference without enhancing tetracycline efflux, it is likely that the greater sensitivity of the wild type reflects potassium depletion upon induction of tetB(L) by tetracycline. That inhibitory effect might outweigh inhibition by tetracycline itself at some levels of tetracycline, transporter, and potassium. The particular conditions of growth could thus provide either positive (high pH, presence of both tetracycline and adequate potassium, high sodium) or negative (neutral pH and limiting potassium) selection pressure for the maintenance of the B. subtilis chromosomal tetB(L) gene. This could explain the presence of this gene in only some B. subtilis strains (11). There have been indications that some tet genes from plasmids might partially complement certain E. coli mutants with defects in potassium transport (43–45), but the mechanism of such effects and their relationship to the usual efflux activity of Tet proteins is unclear.

The capacity of TetB(L) for tetracycline efflux, when expressed from a single copy chromosomal gene in B. subtilis, appears to be modest. In the wild type in the absence of sodium, where optimal preloading with tetracycline occurred, the energy-dependent efflux of tetracycline was briefly evident, but quickly came into equilibrium with the ΔpH-dependent uptake of the antibiotic. This is consistent with the need for amplification in order to support sufficiently fast efflux rates to confer significant resistance to the antibiotic (Fig. 5; 31). The tetracycline/H+ antiport was similarly much lower than that with Na+ as a substrate in the in vitro assay of the E. coli NM81 transformant expressing the cloned tetB(L) gene. It is possible that the assay was not completely optimized for tetracycline as substrate. Since, however, the E. coli transformant also showed only a modest level of tetracycline resistance, it is possible that when TetB(L) and related plasmid tet genes confer high levels of tetracycline resistance in multicopy, it is subsequent to mutations that alter the substrate preference. The data thus far do not allow an evaluation of whether the TetB(L)-mediated tetracycline/H+ antiport is the major mechanism for Na+-dependent pH homeostasis in B. subtilis, as it does in S. pombe (38). Notably, the nhaA gene of E. coli is located near the origin of replication (55), a location that in B. subtilis, may facilitate the amplification of tetB(L) (14). Finally, it would be of interest to assay other monovalent cation/H+ antiporters for tetracycline/H+ antiport and to assay other drug/H+ antiporters, including other categories of tet efflux type genes, for monovalent cation/H+ antiport. The tetracycline/H+ antiport activity of the control E. coli membranes in the current study (Fig. 6) supports the hypothesis that such a capacity may extend beyond genes recognized as tet genes. The genes encoding tetracycline efflux proteins have been recognized as part of a larger group of related transport proteins, the major facilitator family, with homology to other drug/H+ antiporters, e.g. quinolone and antiseptic resistance efflux porters whose mechanism is thought to be antiport (56).

REFERENCES


