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# The *cadC* Gene Product of Alkaliphilic *Bacillus firmus* OF4 Partially Restores $Na^+$ Resistance to an *Escherichia coli* Strain Lacking an $Na^+/H^+$ Antiporter (NhaA)

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**A 5.6-kb fragment of alkaliphilic *Bacillus firmus* OF4 DNA was isolated by screening a library of total genomic DNA constructed in pGEM3Zf(+) for clones that reversed the $Na^+$ sensitivity of *Escherichia coli* NM81, in which the gene encoding an $Na^+/H^+$ antiporter (NhaA) is deleted (E. Padan, N. Maisler, D. Taglicht, R. Karpel, and S. Schuldiner, J. Biol. Chem. 264:20297–20302, 1989). The plasmid, designated pJB22, contained two genes that apparently encode transposition functions and two genes that are apparent homologs of the *cadA* and *cadC* genes of cadmium resistance-conferring plasmid pI258 of *Staphylococcus aureus*. *E. coli* NM81 transformed with pJB22 had enhanced membrane $Na^+/H^+$ antiporter activity that was cold labile and that decreased very rapidly following isolation of everted vesicles. Subclones of pJB22 containing *cadC* as the only intact gene showed identical complementation patterns in vivo and in vitro. The *cadC* gene product of *S. aureus* has been proposed to act as an accessory protein for the $Cd^{2+}$ efflux ATPase (CadA) (K. P. Yoon and S. Silver, J. Bacteriol. 173:7636–7642, 1991); perhaps the alkaliphile CadC also binds $Na^+$ and enhances antiporter activity by delivering a substrate to an integral membrane antiporter. A 6.0-kb fragment overlapping the pJB22 insert was isolated to complete the sequence of the *cadA* homolog. A partial sequence of a region approximately 2 kb downstream of the *cadA* locus shares sequence similarity with plasmids from several gram-positive bacteria. These results suggest that the region of alkaliphile DNA containing the *cadCA* locus is present on a transposon that could reside on a heretofore-undetected endogenous plasmid.**

Alkaliphilic *Bacillus* species growing at a pH higher than 10 maintain a cytoplasmic pH that is much more acidic than that of the external medium by using an $Na^+$ cycle consisting of $Na^+/H^+$ antiporters and a group of $Na^+$/solute symporters (11, 12). The antiporters catalyze the electrogenic exchange of extracellular $H^+$ for intracellular $Na^+$, driven by the proton motive force established by respiration (12, 14). Solute uptake coupled to $Na^+$ entry completes the cycle, which allows cells growing at pH 10.5 to maintain an intracellular pH of approximately 8.3 (7).

The alkaliphile $Na^+$ cycle has been extensively characterized physiologically, and our recent efforts are aimed at identifying and characterizing the genes encoding the antiporter activity. We have used a screen based on the observation of Padan et al. (21) that *Escherichia coli* NM81, which carries a deletion in the *nhaA* $Na^+/H^+$ antiporter gene, does not grow in the presence of high concentrations of $Na^+$ at a pH of $\geq 7.5$. The $Na^+$ sensitivity can be overcome by supplying antiporter genes on plasmids, a fact that recently allowed us to select a clone with a gene encoding an apparent alkaliphile antiporter (10). This gene (*nhaC*) encodes a hydrophobic, 42-kDa protein with predicted modest sequence similarity to the human $Na^+/H^+$ exchanger (25). The clone conferred upon *E. coli* NM81 membrane antiporter activity that was stimulated at an alkaline pH, consistent with a possible role for this protein in pH homeostasis at a high pH. A second gene encoding an alkaliphile antiporter was inferred from the isolation of a transformant of *E. coli* NM81 that may have incorporated an alkaliphile gene distinct from *nhaC* into its genome by recombination with a plasmid from the alkaliphile DNA library (10).

In this report, we describe a third transformant that confers $Na^+$ resistance to *E. coli* NM81 without supplying a structural gene for an $Na^+/H^+$ antiporter. Rather, $Na^+$ resistance is associated with the expression of a small protein normally involved in the efflux system bestowing cadmium resistance (33).

## MATERIALS AND METHODS

**Bacterial strains and growth conditions.** Alkaliphilic *Bacillus firmus* OF4 was grown routinely at pH 10.5 or 7.5 on highly buffered malate-containing media at 30°C as described previously (7). Tests of the cadmium resistance of *B. firmus* OF4 811M required the use of a modified medium to avoid precipitation of the cadmium. The basal medium for growth at pH 7.5 or 9.0 consisted of 50 mM Tris-HCl, 0.1% $(NH_4)_2SO_4$, and 0.1 mM $MgSO_4$. Cadmium resistance at pH 10.2 was monitored in a basal medium containing 50 mM sodium CAPS [3-(cyclohexylamino)-1-propanesulfonic acid] buffer instead of Tris-HCl. All media were supplemented with 50 mM DL-malate, 0.1% yeast extract, and 50 μg of methionine per ml. *E. coli* NM81 (21) was grown at 37°C in LBK medium (21) containing kanamycin (50 μg/ml). Transformants were selected on LBK-kanamycin medium plus ampicillin (100 μg/ml), and 0.4 to 0.7 M NaCl was added to screen for $Na^+$-resistant transformants. *E. coli* JM109 (2) and DH5αMCR (GIBCO-BRL) were used for routine cloning procedures; transformants were selected and maintained on SOB medium (2) containing ampicillin.

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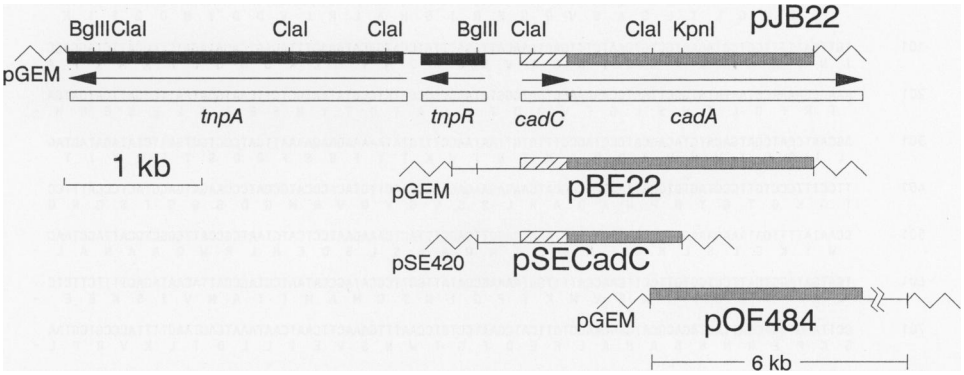


FIG. 1. Organization of the genes on pJB22, its subclones, and overlapping clone pOF484. Arrows indicate the gene length and direction of transcription.

**Preparation of DNA libraries from *B. firmus* OF4.** Chromosomal DNA isolation and preparation of the *Mbo*I-pGEM3Zf(+) (Promega) library were done as described previously (10). A second library designed to isolate overlapping clones was prepared by completely digesting chromosomal DNA from *B. firmus* OF4 with *Cla*I and ligating it into *Acc*I-digested and dephosphorylated pGEM3Zf(+).

**DNA sequencing.** Plasmid DNA was prepared by alkaline lysis and CsCl gradient centrifugation (2) and sequenced by use of an Applied Biosystems 373A DNA sequencer in the DNA Core Laboratory of the Brookdale Center for Molecular Biology at the Mount Sinai School of Medicine. Oligonucleotide primers used for the sequencing were synthesized in the same facility by use of an Applied Biosystems 380B DNA synthesizer. All sequence analyses were done with the Genetics Computer Group Sequence Analysis Software Package (4) run on a VAX 4000-300 computer.

**Assays of $Na^+/H^+$ antiporter activity.** Membrane vesicles prepared from *E. coli* NM81 transformants were used for $Na^+/H^+$ antiporter assays. Everted membrane vesicles were prepared from cells grown in LBK medium at pH 7.5 by the method of Rosen and coworkers (1, 23). Because of the lability of the alkaliphile proteins expressed in *E. coli*, the conditions of Goldberg et al. (5) were modified as described previously (10); i.e., membranes were prepared from fresh cells and assayed immediately after preparation. $Na^+/H^+$ antiporter activity was monitored by the fluorescence quenching of acridine orange and its subsequent reversal upon the addition of NaCl, LiCl, or KCl. The protein concentration was determined by the method of Lowry et al. (15) with egg white lysozyme as a standard.

**Subcloning of pJB22.** pJB22 was digested with *Bgl*II and self-ligated, creating a plasmid, designated pBE22, that contains approximately 180 bp of the extreme 5′ end of the pJB22 insert ligated to 2.5 kb from the 3′ end of the insert. Plasmid pBE22 was then digested with *Bgl*II and *Kpn*I, and the 1.5-kb fragment containing the *cadC* homolog as the only intact gene was cloned into the *trc* promoter expression vector pSE420 (3) to yield pSECadC.

**Isolation of a clone overlapping pJB22.** A 1-kb *Kpn*I-*Pst*I fragment from the 3′ end of pJB22 was isolated from low-melting-point agarose and labelled with $^{32}P$ by use of a random primer system (New England BioLabs) as described by the manufacturer. The *Cla*I-pGEM3Zf(+) library was screened with this probe by colony hybridization. Positive recombinants were selected by use of Southern blots of plasmid minipreparations (2). One recombinant plasmid, designated pOF484, contained a 6-kb insert that was shown to overlap the 3′ end of pJB22 by approximately 1 kb and to extend 5 kb downstream of the *cadA* gene.

**Nucleotide sequence accession number.** The nucleotide sequence data reported here have been deposited in the GenBank Data Bank under accession numbers M90749 (*tnp* locus) and M90750 (*cad* locus).

## RESULTS

**Cloning and characterization of a segment of alkaliphile DNA that partially restores $Na^+$ resistance to *E. coli* NM81.** Cells of *E. coli* strain NM81 transformed with a library of DNA from *B. firmus* OF4 in pGEM3Zf(+) were enriched for $Na^+$-resistant transformants in liquid cultures (0.7 M NaCl in LBK medium) at pH 8.2. Upon subsequent isolation, a transformant, designated pJB22 and containing a 5.6-kb insert, could grow well on plates containing up to 0.4 M NaCl in pH 7.5 medium. Under parallel conditions, NM81 transformed with a control plasmid showed no growth at NaCl concentrations above approximately 0.25 M. The insert was sequenced completely and shown to contain two pairs of open reading frames predicted to be transcribed in opposite directions (Fig. 1). The 5′ pair of open reading frames, which includes a short, complete open reading frame and a long, partial one, is predicted to encode, respectively, homologs of the *tnpR* resolvase and *tnpA* transposase genes of type II transposons of gram-positive bacteria (17). The putative *tnpR* gene would encode a 21-kDa protein with 63% amino acid identity to the resolvase of transposon Tn*917* (26). The partial *tnpA* homolog would encode approximately 85% of a transposase with 53% amino acid sequence identity to the *tnpA* gene product of *Bacillus thuringiensis* transposon Tn*4330* (16).

The second pair of open reading frames is apparently homologous to the *cadC* and *cadA* genes, which make up the cadmium resistance determinant of *S. aureus* plasmid pI258 (19, 20, 27, 30). As with the first pair of open reading frames, the short open reading frame (*cadC*) is complete, whereas only a partial copy of the longer, *cadA* gene is present on the insert (Fig. 1). The deduced product of the *cadC* gene is a 13.9-kDa protein that shares 83% sequence identity with CadC of *S. aureus*. Regions of sequence identity include three motifs that could participate in metal binding (18). The region upstream of *cadC* contains a sequence that is similar to the promoter sequence mapped on pI258 (29) and also

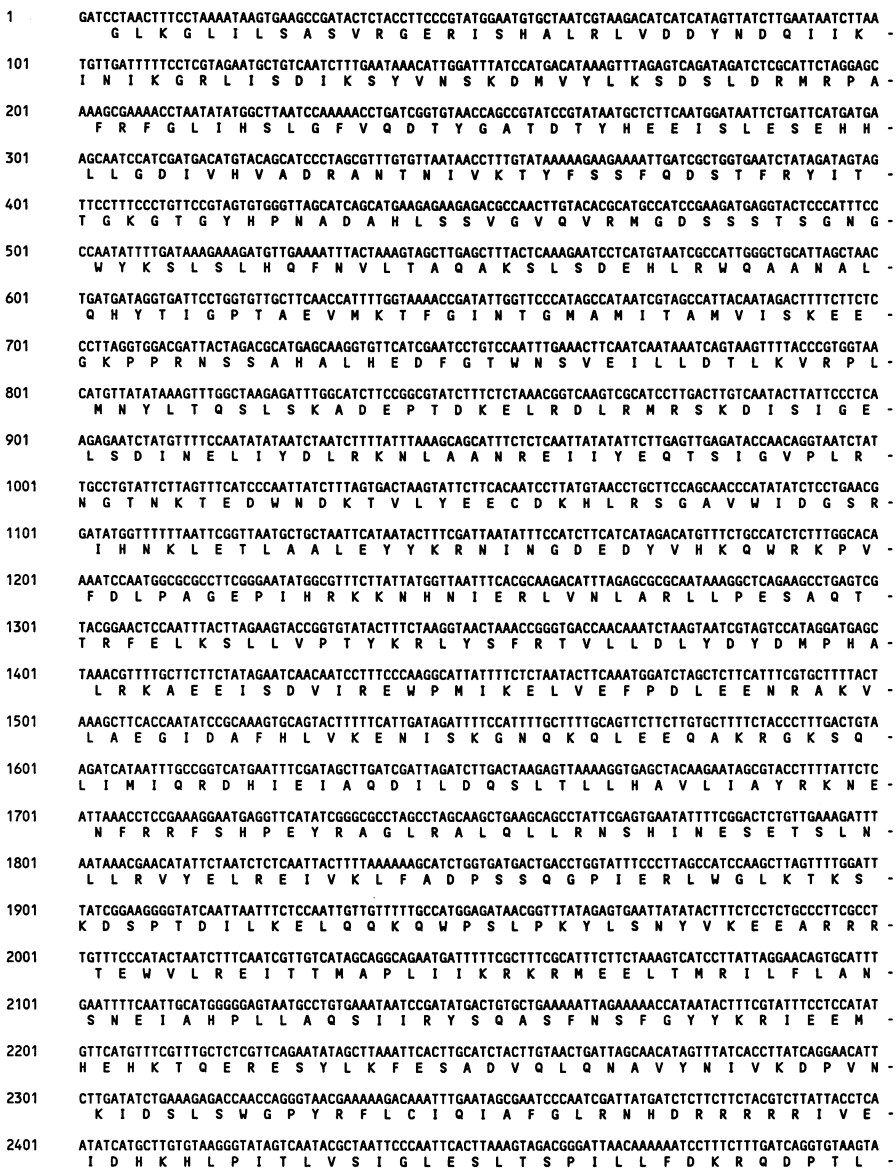

FIG. 2. Nucleotide and deduced amino acid sequences of the pJB22 insert and of the pOF484 region containing the 3′ end of *cadA*. Shaded nucleotides correspond to the promoter that has been mapped for the cadmium resistance determinant of pI258 (29). Arrows indicate an 11-bp inverted repeat analogous to the region of dyad symmetry that has been implicated in the regulation of *S. aureus cad* gene expression (29).

contains an 11-bp inverted repeat that could be involved in regulating the transcription of the cadmium resistance locus. The complete sequence of the putative *cadA* gene was obtained by isolating and partially sequencing an overlapping *Cla*I fragment of *B. firmus* OF4 DNA contained on plasmid pOF484 (Fig. 1). The complete gene for the CadA homolog is predicted to encode a 78-kDa protein sharing 82% amino acid sequence identity with the corresponding gene product of pI258, which is a member of the family of P-type ATPases (29). The complete nucleotide sequence of the pJB22 insert and the additional *cadA* gene 3′ region is shown in Fig. 2, along with the putative gene products. Although data are not shown, *B. firmus* OF4 was found to be completely resistant to $CdCl_2$ up to a concentration of 1 mM.

An additional nucleotide sequence for regions approximately 2 and 5 kb downstream of the *cadA* gene has been obtained from pOF484. The sequence is not predicted to encode a protein with significant similarity to any protein in the data bases (GenBank release 71.0, PIR-Protein release 31.0), but there is similarity at the DNA level to plasmids, including Tn*4330*-containing plasmid pG12 (26), from several gram-positive bacteria (data not shown). This result, together with the close juxtaposition of transposase and cadmium resistance determinants, suggests that alkaliphilic *B. firmus* OF4 may contain an endogenous plasmid that harbors a transposon encoding these functions. Earlier findings had indicated an instability of one $Na^+/H^+$ antiporter gene (10) and the generation of genetic variants of *B. firmus* RAB and OF4 (6, 13) that could be related to an endogenous transposon. Also, the presence of such a transposon may have facilitated the transposition by Tn*925* reported in earlier studies (8).

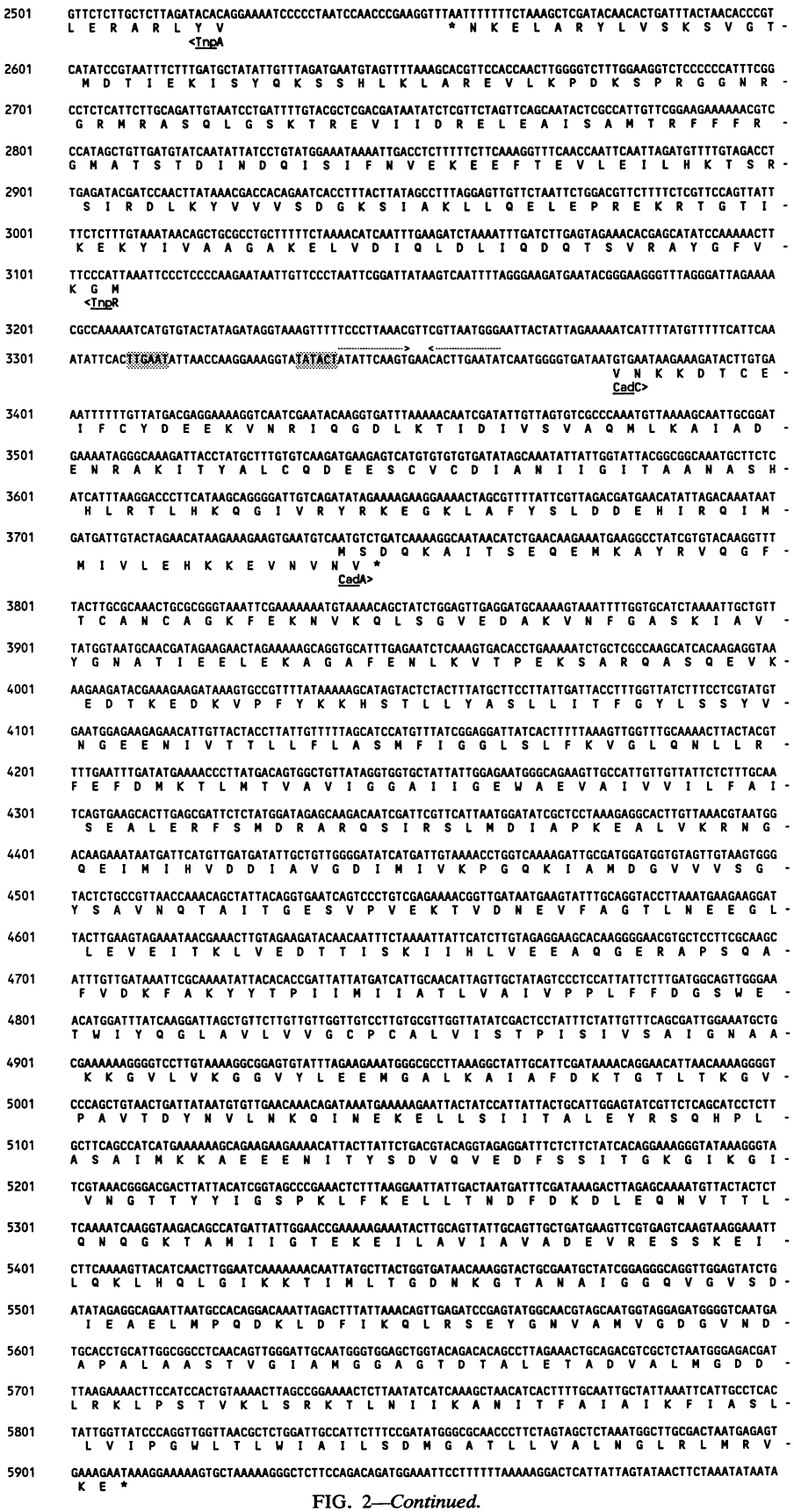


FIG. 2—*Continued.*

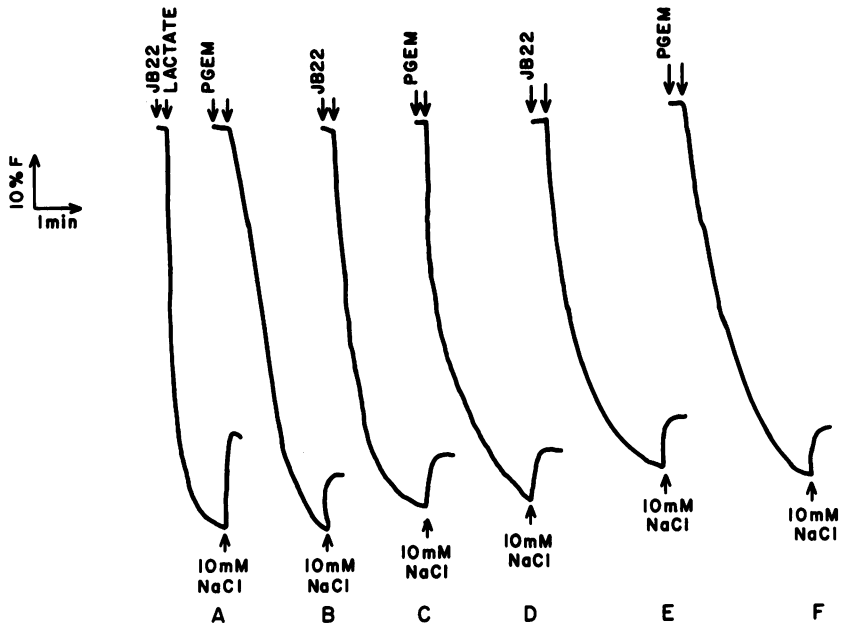


FIG. 3. Antiporter activity of everted membrane vesicles from *E. coli* NM81 transformed with either pJB22 or the pGEM7Zf(+) control plasmid. Antiporter activity was measured by monitoring the fluorescence (F) of acridine orange as described in Materials and Methods. As indicated by the second arrow at the top of the tracing, the energy donor Tris–D-lactate was added to initiate quenching. After the addition of the appropriate antiporter substrate (lower arrows), antiporter activity was measured as the reversal of quenching. All assays were performed at pH 8.0. The precise conditions were as follows: A and B, vesicles made at room temperature and assayed immediately thereafter; C and D, vesicles assayed after being kept on ice for 1 h; and E and F, vesicles assayed after 2 h at room temperature.

**Enhancement of the $Na^+/H^+$ antiporter activity of *E. coli* NM81 by pJB22 and its subclones.** The mechanism by which pJB22 conferred $Na^+$ resistance to *E. coli* NM81 was investigated by assaying the $Na^+/H^+$ antiporter activity of membrane vesicles prepared from *E. coli* NM81 containing either pJB22 or a control plasmid [pGEM7Zf(+)]. As shown in Fig. 3A and B, membranes from the pJB22 transformant showed significantly enhanced $Na^+/H^+$ antiporter activity compared with the plasmid control, on the basis of the reversal of acridine orange quenching resulting from the

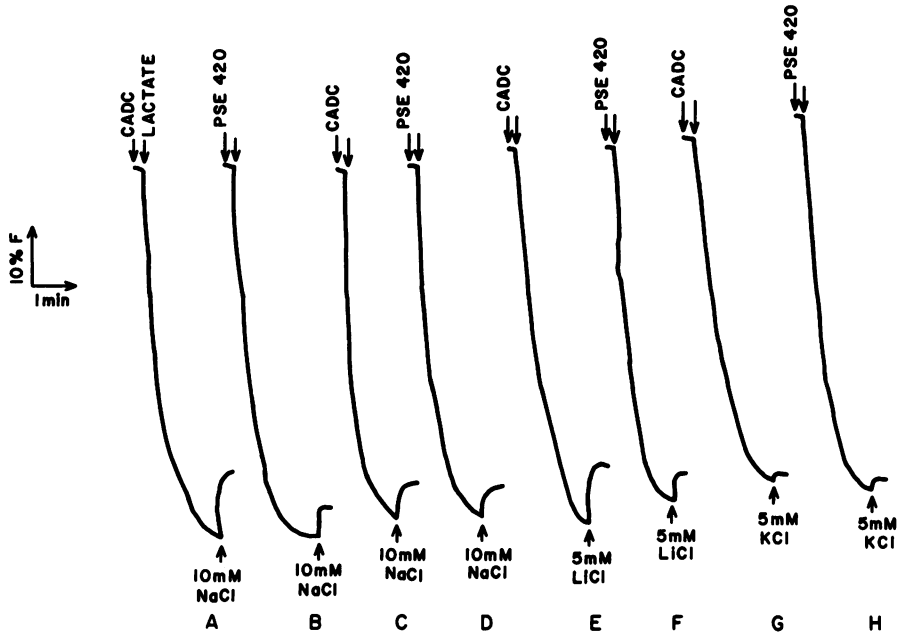


FIG. 4. Antiporter activity of everted membrane vesicles prepared from *E. coli* NM81 transformed with either pSECadC or the pSE420 control plasmid. Assays were done as described in the legend to Fig. 3. The conditions for each assay were as follows: A, B, E, F, G, and H, vesicles prepared at room temperature and assayed immediately thereafter; C and D, vesicles assayed after being kept on ice for 1 h.

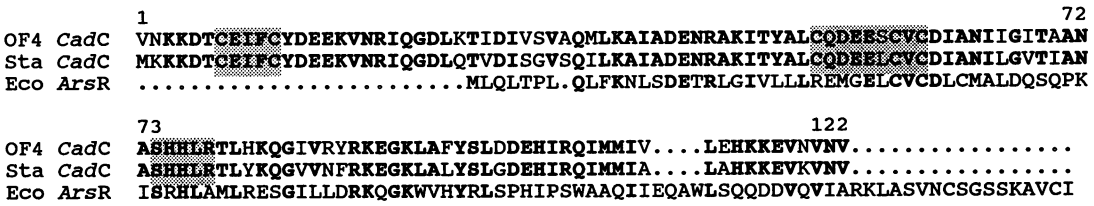


FIG. 5. Alignment of the deduced amino acid sequences of the *B. firmus* OF4 and *S. aureus* (Sta) *cadC* genes and the *E. coli* (Eco) *arsR* gene. Shaded areas are potential cation-binding domains (18).

addition of 10 mM NaCl. The enhancement of activity was very unstable, requiring that vesicles be used immediately after preparation and be kept at room temperature during the assay period. Short incubations on ice were inhibitory (Fig. 3C and D), as were prolonged incubations at room temperature (Fig. 3E and F). These properties had not been observed in earlier studies with membranes from transformants of *E. coli* that had alkaliphile *nhaC*-encoded antiporter activity (10).

Subclones of pJB22 were created to determine the region of the insert responsible for conferring $Na^+$ resistance and enhanced $Na^+/H^+$ antiporter activity. Initially, a subclone (pBE22) containing only the cadmium locus was shown to confer enhanced $Na^+/H^+$ antiporter activity upon membranes of *E. coli* NM81 (data not shown). A 1.5-kb fragment of this subclone, containing the intact *cadC* homolog and approximately one-third of the *cadA* gene, was cloned into the *trc* promoter expression vector pSE420 so that the *cadC* gene was under the control of its own promoter as well as the isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible *trc* promoter. This subclone, designated pSECadC (Fig. 2), also conferred enhanced $Na^+/H^+$ antiporter activity upon membranes of *E. coli* NM81 (Fig. 4A and B). As was the case with pJB22, the enhancement of activity was cold labile and decreased with time (Fig. 4C and D). As shown in Fig. 4E to H, the addition of LiCl but not KCl also resulted in the enhancement of $Na^+/H^+$ antiporter activity.

## DISCUSSION

Previous studies have shown that the $Na^+$ sensitivity of the *E. coli nhaA* deletion strain NM81, which lacks an $Na^+/H^+$ antiporter, can be complemented by antiporter genes on multicopy plasmids (5, 10, 21, 22). Here we show that $Na^+$ resistance and enhanced antiporter activity can also be restored to *E. coli* NM81 by a plasmid that does not contain a structural gene encoding an $Na^+/H^+$ antiporter but that does contain a gene, *cadC*, encoding a putative cation-binding protein (33). The *cadC* gene conferred reproducible and significant $Na^+$ resistance upon antiporter-deficient NM81 at up to 0.4 M NaCl and pH 7.5, whereas the *nhaC* gene conferred resistance at up to 0.6 M NaCl and pH 7.5 (10). The *cadC* gene product, along with CadA, the membrane-bound homolog of the P-type ATPases, makes up the cadmium resistance locus of *S. aureus* plasmid pI258 (18, 33). Yoon and colleagues (32, 33) have shown that the two proteins function in concert as a $Cd^{2+}$ efflux system by using energy derived from ATP hydrolysis to pump $Cd^{2+}$ out of the cell. We were therefore aware of the possibility that the products of the genes encoded on pJB22 might act as an $Na^+$ efflux pump and thereby confer $Na^+$ resistance upon *E. coli* NM81, particularly in light of a recent report (9) that the yeast *ENA1* gene, encoding a P-type ATPase, could complement an $Li^+$-sensitive yeast strain. However, the truncated *cadA* gene contained on pJB22 would encode a protein that would not have the highly conserved "hinge" region shown by others to be required for E1-E2 conformational switching of P-type ATPases (28) and therefore would not be predicted to function. More importantly, subclone pSE-CadC, which lacks the region that would encode the putative transduction and ATP-binding domains of CadA but has the entire *cadC* gene, provided enhancement of $Na^+/H^+$ antiporter activity that was identical to that provided by pJB22.

CadC is required for cadmium resistance in *S. aureus* (33). On the basis of their studies of the induction of *cadA* and *cadC* gene expression, Yoon and Silver (33) concluded that CadC does not have a regulatory role, despite the shared sequence similarity with the *trans*-acting regulatory protein ArsR of the arsenical resistance determinant of plasmid R773 (24, 31) (Fig. 5). The predicted amino acid sequence of *S. aureus* CadC reveals three possible cation-binding motifs (18), leading to the suggestion that it functions to sequester intracellular $Cd^{2+}$ and deliver it to the CadA efflux pump. By analogy, the *B. firmus* OF4 homolog of CadC, which has identical cation-binding domains (Fig. 5) may, when overexpressed in *E. coli* NM81, bind intracellular $Na^+$ and may in addition deliver it to the residual $Na^+/H^+$ antiporter encoded by *nhaB* (21, 22) through presumably nonspecific interactions at the cytoplasmic surface of the membrane. Such activities would account for both the enhanced resistance to $Na^+$ conferred upon *E. coli* NM81 and the enhanced membrane $Na^+/H^+$ antiporter activity. The relative importance of $Na^+$ sequestration versus substrate delivery to the integral membrane antiporter will be possible to evaluate once mutants with deletions in both *nhaA* and *nhaB* are available. The current in vitro data support the conclusion that a binding protein may enhance antiporter activity through a labile interaction that is cold sensitive. We hypothesize that this interaction enhances the availability of $Na^+$ to the antiporter.

## ACKNOWLEDGMENT

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## REFERENCES

1. **Ambudkar, S. V., G. W. Zlotnick, and B. P. Rosen.** 1984. Calcium efflux from *Escherichia coli*. Evidence for two systems. J. Biol. Chem. **259**:6142–6146.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. A. Smith, J. G. Seidman, and K. Struhl (ed.).** 1987. Current protocols in molecular biology. John Wiley & Sons, Inc., New York.
3. **Brosius, J.** Compilation of superlinker vectors. Methods Enzymol., in press.
4. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic

Acids Res. **12**:387–395.
5. **Goldberg, E. B., T. Arbel, J. Chen, R. Karpel, G. A. Mackie, S. Schuldiner, and E. Padan.** 1987. Characterization of a $Na^+/H^+$ antiporter gene of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **84**:2615–2619.
6. **Guffanti, A. A.** Unpublished data.
7. **Guffanti, A. A., and D. B. Hicks.** 1991. Molar growth yields and bioenergetic parameters of extremely alkaliphilic *Bacillus* species in batch cultures, and growth in a chemostat at pH 10.5. J. Gen. Microbiol. **137**:2375–2379.
8. **Guffanti, A. A., P. G. Quirk, and T. A. Krulwich.** 1991. Transfer of Tn*925* and plasmids between *Bacillus subtilis* and alkaliphilic *Bacillus firmus* OF4 during Tn*925*-mediated conjugation. J. Bacteriol. **173**:1686–1689.
9. **Haro, R., B. Garciadeblas, and A. Rodriguez-Navarro.** 1991. A novel P-type ATPase from yeast involved in sodium transport. FEBS Lett. **291**:189–191.
10. **Ivey, D. M., A. A. Guffanti, J. S. Bossewitch, E. Padan, and T. A. Krulwich.** 1991. Molecular cloning and sequencing of a gene from alkaliphilic *Bacillus firmus* OF4 that functionally complements an *Escherichia coli* strain carrying a deletion in the *nhaA* $Na^+/H^+$ antiporter gene. J. Biol. Chem. **266**:23483–23489.
11. **Krulwich, T. A., and A. A. Guffanti.** 1989. Alkalophilic bacteria. Annu. Rev. Microbiol. **43**:435–463.
12. **Krulwich, T. A., and A. A. Guffanti.** 1989. The $Na^+$ cycle of extreme alkalophiles: a secondary $Na^+/H^+$ antiporter and $Na^+$/solute symporters. J. Bioenerg. Biomembr. **21**:663–677.
13. **Krulwich, T. A., A. A. Guffanti, M. Y. Fong, L. Falk, and D. B. Hicks.** 1986. Alkalophilic *Bacillus firmus* RAB generates variants which can grow at lower $Na^+$ concentrations than the parental strain. J. Bacteriol. **165**:884–889.
14. **Krulwich, T. A., A. A. Guffanti, and D. Seto-Young.** 1990. pH homeostasis and bioenergetic work in alkalophiles. FEMS Microbiol. Rev. **6**:271–278.
15. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
16. **Mahillon, J., and D. Lereclus.** 1988. Structural and functional analysis of Tn4430: identification of an integrase-like protein involved in the cointegrate-resolution process. EMBO J. **7**:1515–1526.
17. **Murphy, E.** 1989. Transposable elements in gram-positive bacteria, p. 269–288. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
18. **Nies, D. H.** 1992. Resistance to cadmium, cobalt, zinc, and nickel in microbes. Plasmid **27**:17–28.
19. **Novick, R. P., and C. Roth.** 1968. Plasmid-linked resistance to inorganic salts in *Staphylococcus aureus*. J. Bacteriol. **95**:1335–1342.
20. **Nucifora, G., L. Chu, T. K. Misra, and S. Silver.** 1989. Cadmium resistance from *Staphylococcus aureus* plasmid pI258 *cad*A gene results from a cadmium-efflux ATPase. Proc. Natl. Acad. Sci. USA **86**:3544–3548.
21. **Padan, E., N. Maisler, D. Taglicht, R. Karpel, and S. Schuldiner.** 1989. Deletion of *ant* in *Escherichia coli* reveals its function in adaptation to high salinity and an alternative $Na^+/H^+$ antiporter system(s). J. Biol. Chem. **264**:20297–20302.
22. **Pinner, E., E. Padan, and S. Schuldiner.** 1992. Cloning, sequencing, and expression of the *nhaB* gene, encoding a $Na^+/H^+$ antiporter in *Escherichia coli*. J. Biol. Chem. **267**:11064–11068.
23. **Rosen, B. P.** 1986. Ion extrusion systems in *Escherichia coli*. Methods Enzymol. **125**:328–336.
24. **San Francisco, M. J. D., C. L. Hope, J. B. Owolabi, L. S. Tisa, and B. P. Rosen.** 1990. Identification of the metalloregulatory element of the plasmid-encoded arsenical resistance operon. Nucleic Acids Res. **18**:619–624.
25. **Sardet, C., A. Franchi, and J. Pouyssegur.** 1989. Molecular cloning, primary structure, and expression of the human growth factor-activatable $Na^+/H^+$ antiporter. Cell **56**:271–280.
26. **Shaw, J. H., and D. B. Clewell.** 1985. Complete nucleotide sequence of macrolide-lincosamide-streptogramin B resistance transposon Tn*917* in *Staphylococcus faecalis*. J. Bacteriol. **164**:782–796.
27. **Silver, S.** 1992. Plasmid-determined metal resistance mechanisms: range and overview. Plasmid **27**:1–3.
28. **Silver, S., G. Nucifora, L. Chu, and T. K. Misra.** 1989. Bacterial resistance ATPases: primary pumps for exporting toxic cations and anions. Trends Biochem. Sci. **14**:76–80.
29. **Silver, S., and M. Walderhaug.** 1992. Gene regulation of plasmid- and chromosome-determined inorganic ion transport in bacteria. Microbiol. Rev. **56**:195–228.
30. **Smith, K., and R. P. Novick.** 1972. Genetic studies on plasmid-linked cadmium resistance in *Staphylococcus aureus*. J. Bacteriol. **112**:761–772.
31. **Wu, J., and B. P. Rosen.** 1991. The ArsR protein is a trans-acting regulatory protein. Mol. Microbiol. **5**:1331–1336.
32. **Yoon, K. P., T. K. Misra, and S. Silver.** 1991. Regulation of the *cadA* cadmium resistance determinant of *Staphylococcus aureus* plasmid pI258. J. Bacteriol. **173**:7643–7649.
33. **Yoon, K. P., and S. Silver.** 1991. A second gene in the *Staphylococcus aureus cadA* cadmium resistance determinant of plasmid pI258. J. Bacteriol. **173**:7636–7642.