An *Escherichia coli* Chromosomal *ars* Operon Homolog Is Functional in Arsenic Detoxification and Is Conserved in Gram-Negative Bacteria

CAROLINE DIORIO, JIE CAI, JOY MARMOR, ROWEN SHINDER, AND MICHAEL S. DU BOW*

Department of Microbiology and Immunology, McGill University, Montreal, Quebec, Canada H3A 2B4

Received 8 November 1994/Accepted 13 February 1995

Arsenic is a known toxic metalloid, whose trivalent and pentavalent ions can inhibit many biochemical processes. Operons which encode arsenic resistance have been found in multicopy plasmids from both gram-positive and gram-negative bacteria. The resistance mechanism is encoded from a single operon which typically consists of an arsenite ion-inducible repressor that regulates expression of an arsenite reductase and inner membrane-associated arsenite export system. Using a *lacZ* transcriptional gene fusion library, we have identified an *Escherichia coli* operon whose expression is induced by cellular exposure to sodium arsenite at concentrations as low as 5 μg/liter. This chromosomal operon was cloned, sequenced, and found to consist of three cistrons which we named *arsR*, *arsB*, and *arsC* because of their strong homology to plasmid-borne *ars* operons. Mutants in the chromosomal *ars* operon were found to be approximately 10- to 100-fold more sensitive to sodium arsenate and arsenite exposure than wild-type *E. coli*, while wild-type *E. coli* that contained the operon cloned on a ColE1-based plasmid was found to be at least 2- to 10-fold more resistant to sodium arsenate and arsenite. Moreover, Southern blotting and high-stringency hybridization of this operon with chromosomal DNAs from a number of bacterial species showed homologous sequences among members of the family Enterobacteriaceae, and hybridization was detectable even in *Pseudomonas aeruginosa*. These results suggest that the chromosomal *ars* operon may be the evolutionary precursor of the plasmid-borne operon, as a multicopy plasmid location would allow the operon to be amplified and its products to confer increased resistance to this toxic metalloid.

### MATERIALS AND METHODS

**Bacterial strains and phages.** The following bacterial strains used were all derivatives of *E. coli K-12*: *E. coli* 40 (*Δpro-lac-mdh* *apv*), *E. coli* BU5029 (a recA mutant derivative of strain 40), and those described (including sources) by Autexier and DuBow (1). Phages MudI and P1vir were kind gifts of M. Casada-\_ban (University of Chicago) and R. Stewart (McGill University), respectively.

**DNA manipulations.** All restriction endonuclease hydrolys and DNA ligations were performed as described by Tolias and DuBow (43). DNA sequencing

---

* Corresponding author. Mailing address: Department of Microbiology and Immunology, McGill University, 3775 University St., Montreal, Quebec, Canada H3A 2B4. Phone: (514) 398-3076, Fax:(514) 398-7052. Electronic mail address: indw@musicb.mcgill.ca.
of both strands (see Fig. 2A) was performed by the dideoxy DNA sequencing method with single-stranded DNAs from cloned fragments in plasmids pUC118 and pUC119 (44) by using the Sequenase version 2.0 kit from United States Biochemicals. Southern blotting and hybridization, as well as isolation of total cellular DNA, were performed according to the method of Autexier and Dubow (1), while PI transduction was done according to the method of Miller (25). DNA was isolated from stationary-phase cells grown in Luria-Bertani (LB) (25) broth (E. coli 40 and Pseudomonas aeruginosa PA01) or nutrient broth (Difco Laboratories) (Shigella sonnei, Citrobacter freundii, Enterobacter cloacae, Salmonella arizonae, Erwinia carotovora, and Klebsiella pneumoniae) at 37°C, except for E. coli 40, P. aeruginosa PA01, and S. arizonae, which were grown at 32°C. For Southern blotting, 10 μg of total cellular DNA was digested with the appropriate restriction enzyme and blotted to nylon (Hybond-N; Amersham) membranes following electrophoresis through 0.75% agarose gels (31). Membranes were probed with 2 × 106 to 4 × 107 cpm of an α-32P-labelled 1,188-bp EcoRV (bp 1664 to 2852 [see Fig. 2A]) or a 587-bp NdeI-EcoRV (bp 1077 to 1664 [see Fig. 2A]) DNA fragment (prepared by the random priming method [31]) per ml under high-stringency conditions (1). After being washed, membranes were exposed to Agfa Curity RFI X-ray film.

Construction of strains LF20001 and LF20018. lacZ fusions to chromosomal genes were constructed by infecting E. coli 40 with MudI (amp' lac') bacteriophages as described by Casadaban and Cohen (6). The resultant clones were picked to a master plate and replicated on LB agar plates that contained ampicillin and 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside (X-Gal; Research Organics, Inc.) plus increasing concentrations of sodium arsenate (0.1 to 10 μg/ml). One clone, which became blue when it was grown in the presence of sodium arsenate and remained white in its absence, was named LF20001 and its plasmid was designated pJS29 (Fig. 1). The cloned chromosomal DNA adjacent to the right end of the MudI insertion was isolated and used as a probe to identify a 15-kb PstI fragment from the chromosome of E. coli 40. The 15-kb fragment was cloned into pBR322 (digested with PstI) to yield plasmid pJC076 (Fig. 1) by standard procedures (31). A 3-kb NsiI-BglII fragment from pJC076 passed the site of MudI insertion in strain LF20001 was subcloned (plasmid pJC073) and completely sequenced.

β-Galactosidase assays. β-Galactosidase assays were performed as described by Miller (25) by the chloroform-sodium dodecyl sulfate cell lysis procedure. Cells were grown to an A550 of 0.4 in LB broth at 32°C and exposed to various arsenic and antimony compounds, and aliquots were removed for β-galactosidase assays after 30 min.

Arsenic sensitivity tests. The sensitivities of E. coli strains to trivalent and pentavalent arsenic ions were determined by preparing petri plates that contained LB agar and various concentrations of sodium arsenate and sodium arsenic. Overnight cultures of E. coli strains grown in LB broth were diluted in fresh LB broth and grown at 32°C to an A550 of approximately 0.4. Then, cells were diluted 104-fold in LB broth, and 0.1 ml of these dilutions was spread (in triplicate) on different agar plates. Petri dishes were incubated at 32°C overnight, and then colonies were counted.

Nucleotide sequence accession number. The nucleotide sequence reported here has been submitted to the EMBL database, and it has been assigned accession number X80057.

RESULTS

Discovery of a chromosomal ars operon homolog. A collection of lacZ transcriptional gene fusions was prepared by using E. coli 40 and the MudI bacteriophage (6). In order to identify any gene whose transcription is specifically affected by arsenic salts, this collection of clones was replicated on petri dishes in the absence and presence of various concentrations of sodium arsenate and the β-galactosidase indicator substrate X-Gal. A single clone which formed blue colonies on petri plates that contained sodium arsenate and white colonies in its absence was identified. This clone, designated strain LF20001, was isolated for further study. A P1vir transductant (25) of E. coli LF20001 into E. coli 40 and selection on LB plates that contained ampicillin. The resultant Ap' clone (E. coli LF20018) was tested for arsenic induction of β-galactosidase, and the location of the MudI prophage was determined by Southern blotting and hybridization, with the lacZ gene as the probe.

Isolation of the arsenic-inducible operon. To isolate the proximal portion of the arsenate-inducible operon, a lac operon-Mu868E. coli DNA fragment was cloned from strain LF20001 via isolation of total cellular DNA (13), cleavage with BglII, ligation into BamHI-cleaved pBR322 DNA, and transformation into E. coli BL2101. One colony, which developed a blue color on LB agar with ampicillin and X-Gal (because of amplification of the lac operon), was selected, and its plasmid was designated pJS29 (Fig. 1). The cloned chromosomal DNA adjacent to the right end of the MudI insertion was isolated and used as a probe to identify a 15-kb PstI fragment from the chromosome of E. coli 40. The 15-kb fragment was cloned into pBR322 (digested with PstI) to yield plasmid pJC076 (Fig. 1) by standard procedures (31). A 3-kb NsiI-BglII fragment from the 15-kb PstI fragment cloned into the PstI site of pBR322 (pJC076) and its plasmid was designated pJS29 (Fig. 1). The cloned chromosomal DNA (spanning the MudI junction) cloned into the BamHI site of pBR322 was subcloned in strain LF20018 to test for arsenic sensitivity. The DNA adjacent to the MudI prophage was mapped by Southern blotting (with the lac operon as the probe), cloned, and used as a probe to map and isolate the DNA sequences that flank the MudI insertion site from the chromosome of E. coli 40 (Fig. 1). By hybridization of the
chromosomal DNA (12) in plasmid pJC103 to the Kohara et al. phage set (22), it was determined that the arsenate-inducible gene was located at 77.5 min on the *E. coli* genetic map. A total of 2.973 kb of DNA was sequenced (EMBL accession number X80057) (Fig. 2A) from plasmid pJC103 and used to scan databases with the University of Wisconsin Genetics Computer Group sequence analysis software package. It was found that this region of the chromosome is highly homologous to the arsenic-inducible *ars* operons of plasmid isolates from *E. coli* (7, 32, 48), *Staphylococcus aureus* (17), and *Staphylococcus xylosus* (30) (Fig. 2B); thus, because of its homology and arsenate inducibility, it was designated *ars*. This chromosomal *ars* operon was found to consist of three cistrons, which we have named *arsR*, *arsB*, and *arsC* because of strong homology to the plasmid-borne *ars* operons. The *arsR* cistron is 77.0% homologous (at the protein level) to the same cistron in the *ars* operon isolated from plasmid R773 of *E. coli*, while the *arsB* and *arsC* cistrons are 90.7 and 94.3% homologous, respectively, with this operon. Weaker, though still significantly homologous, are the plasmid-encoded ArsR, ArsB, and ArsC proteins of *S. aureus* and *S. xylosus* (Fig. 2B). The location of the MudI prophage in *E. coli* LF20001 was found to be in the *arsB* gene, 799 bp downstream from the ATG start codon. Transcription of the inserted promoterless lac operon would occur from an upstream promoter (Fig. 2A), presumably located in a position similar to that of the *ars* operon in plasmid R773 (47).

**Effects of various arsenic and antimony compounds on *ars* gene expression.** The plasmid-borne *ars* operons are inducible by various toxic arsenic and antimony compounds (38). In order to measure induction of expression of the chromosomal *ars* operon by these compounds, *E. coli* LF20001 was grown...
in LB broth and exposed to various compounds, and β-galactosidase activity was quantified. Maximal levels of β-galactosidase activity were reached at approximately 60 min after exposure of strain LF20001 to sodium arsenate at final concentrations that ranged from 1 to 10 μg/ml (data not shown). When strain LF20001 was exposed to increasing concentrations of sodium arsenite, induction of gene expression was detectable at 30 min postexposure and 5 μg/liter, with maximal induction observed at 1 μg/ml (Fig. 3). Sodium arsenate, the pentavalent (and less toxic) form of arsenic, did not induce ars operon expression at 30 min postexposure and 5 μg/liter. However, higher concentrations (100 and 1,000 μg/liter) were able to induce expression of the arsB::lacZ fusion. Antimony (as antimony oxide), located just below arsenic in the periodic table, was also found to induce ars operon expression, as it does for plasmid-borne ars operons (34). However, cacodylic acid, a relatively nontoxic pesticide which contains arsenic in an organic formulation (3), was unable to induce expression of the arsB::lacZ fusion. Antimony (as antimony oxide), located just below arsenic in the periodic table, was also found to induce ars operon expression, as it does for plasmid-borne ars operons (34).

A functional role for the ars operon in protection from arsenic toxicity. In order to determine if the chromosomal ars operon plays a functional role in arsenic detoxification, clones with (E. coli 40) and without (E. coli LF20001 and LF20018) a functional ars operon were tested in identical genetic backgrounds for their growth in arsenic-containing LB media. Increasing concentrations of sodium arsenite or sodium arsenate were added to LB agar on petri dishes, and the colony-forming capacities of various strains were examined after overnight growth. The 50% lethal concentrations of sodium arsenite and sodium arsenate for E. coli 40 were found to be between 200 and 2,000 μg/ml (Fig. 4), and similar results were obtained for E. coli MG1655 (data not shown). Disruption of the chromosomal ars operon by MudI insertion was found to increase the sensitivity of E. coli 40 to sodium arsenite (Fig. 4A) and sodium arsenate (Fig. 4B) by approximately 10- to 100-fold (Fig. 4A). However, when the complete ars operon, cloned on a multicopy plasmid (pJC103), was introduced into wild-type E. coli 40, resistance to sodium arsenite (Fig. 4A) and sodium arsenate (Fig. 4B) increased by at least 3- to 10-fold, though the absolute levels of resistance were somewhat lower than those observed for E. coli that contained the arsRDABC operon of plasmid R773 (34). The ability of E. coli arsB mutant strains (LF20001 and LF20018) to survive at arsenic levels which
induce ars operon expression (0.01 to 1 μg/ml) may be due to other cellular detoxification mechanisms, such as those provided by glutathione and thioredoxin (11, 15, 16).

**Sequences homologous to the chromosomal ars operon are highly conserved.** Because of the high degree of homology between the protein products of the _E. coli_ chromosomal ars operon and those found on plasmids from both gram-negative and gram-positive bacteria, we sought to determine if the chromosomal operon was conserved at the DNA level (and thus, possibly the progenitor of plasmid-based arsenic resistance determinants). DNA was isolated from a number of plasmid-free, gram-negative bacterial species (1), hydrolyzed with restriction enzymes, and Southern blotted after agarose gel electrophoresis. After hybridization with an _E. coli_ ars-specific probe, sequences that were homologous to the _E. coli_ chromosomal ars operon were found in all of the enterobacterial species examined. Moreover, homologous sequences to the ars operon were detected in the nonenterobacterial species _P. aeruginosa_ (Fig. 5). This high degree of evolutionary conservation at the DNA level strongly reinforces notions that the chromosomal ars operon is functionally important and that its chromosomal presence is not of recent origin.

**DISCUSSION**

We have discovered a functional, arsenic-inducible operon in the chromosome of _E. coli_, with homologous sequences detectable in many other gram-negative bacterial species. This operon displays strong homology, both in protein sequence and genetic organization, with plasmid-borne arsenic detoxification operons. During the later stages of this work, continued sequencing of the _E. coli_ genome also uncovered this chromosomal ars operon homolog, though no functional studies were performed (40). The names _arsE_, _arsF_, and _arsG_ were given to these three homologous cistrons, but _arsR_, _arsB_, and _arsC_ more accurately reflect their evolutionary relatedness and probable function(s). Thus, it is likely that the chromosomal ars operon is organized as a single transcription unit that is regulated by the arsenic- and antimony-inducible ArsR repressor. Moreover, the structural genes of the chromosomal ars operon appear to encode an arsenate reductase (_arsC_) and an arsenite-specific efflux system (_arsB_). The apparent strong evolutionary conservation of chromosomal ars determinants also suggests that this operon may be the progenitor of plasmid-borne ars operons. The origins of many plasmid-borne resistance determinants have not yet been elucidated. However, it is known that β-lactamases are also highly conserved, whether their lo-
cations are chromosomal or on plasmids (26, 33). In addition, hemolysins show similarly strong evolutionary conservation (2, 10). More recently, however, a chromosomal homolog of a plasmid-borne copper resistance operon has been found in *Pseudomonas syringae* (23). To our knowledge, its evolutionary conservation has not been determined.

It has been proposed that the structure of the plasmid-borne, ATP-driven arsenic efflux pump, made up of the ArsA and ArsB proteins, may be structurally related to the multiple drug resistance ATP-driven efflux pump found amplified in mammalian cancer cells (36, 37, 49). During chemotherapy of cancer patients, cells become resistant to anti-cancer chemotherapeutic agents by amplification of the number of copies of the multiple drug resistance gene and thus overexpression of the multiple drug resistance pump (28). In analogous fashion, amplification of the chromosomal *ars* operon, by its presence on multicopy plasmids, should allow increased resistance to cellular exposure to toxic arsenic salts. In this regard, we found that the presence of this operon in pBR322 (pIC103), under its own regulation, conferred at least a 5- to 10-fold increase in the resistance of *E. coli* to arsenate or arsenite exposure.

A lacZ fusion to the chromosomal *ars* operon was found to be induced by arsenic compounds at concentrations that reflected their relative toxicities (arsenate < arsenite). Moreover, antimony oxide also induced expression of the *ars* operon (28). In an analogous fashion, the presence of this operon in *E. coli* provided the relative toxicities of arsenic and antimony compounds (28). In an analogous fashion, the presence of this operon in *E. coli* provided the relative toxicities of arsenic and antimony compounds (28). More recently, however, a chromosomal homolog of a hemolysin shows similarly strong evolutionary conservation (2, 16). Expression of this outer membrane protein of *E. coli* is regulated by metal ions. Appl. Environ. Microbiol. 57:2255–2259.

**REFERENCES**

1. Autesier, C., and M. S. DuBow. 1992. The *Escherichia coli* Mu/Δ108 phage resistance operon (mcr) is transcribed and evolutionarily conserved among the *Enterobacteriaceae*. Gene 114:13–18.

2. Baba, K., H. Shirai, A. Terai, K. Kumagai, Y. Takeda, and M. Nishibuchi. 1991. Similarity of the *tdh* gene-bearing plasmids of *Vibrio cholerae* non-01 and *Vibrio parahaemolyticus*. Microbiol. Pathog. 16:61–70.

3. Brannon, J. M., and W. H. Patrick, Jr. 1987. Fixation, transformation, and mobilization of arsenic in sediments. Environ. Sci. Technol. 21:450–459.

4. Broër, S., G. Ji, A. Broër, and S. Silver. 1993. Arsenic efflux governed by the arsenic resistance determinant of *Staphylococcus aureus* plasmid pI258. J. Bacteriol. 175:3480–3485.

5. Carter, N. S., and A. H. Fairlamb. 1993. Arsenical-resistant tyropanosomes lack an unusual adenosine transporter. Nature (London) 361:173–176.

6. Casadaban, M. J., and S. N. Cohen. 1979. Lac operon genes fused to exogenous promoters in one step using a Mu-lac bacteriophage: *in vivo* probe for transcriptional control sequences. Proc. Natl. Acad. Sci. USA 76:4530–4533.

7. Chen, C.-M., T. K. Misra, S. Silver, and B. P. Rosen. 1986. Nucleotide sequence of the structural genes for an anion pump: the plasmid-encoded arsenical resistance operon. J. Biol. Chem. 261:15030–15038.

8. Corbiser, P., G. Ji, G. Nuyts, M. Mergeay, and S. Silver. 1993. *luxAB* gene fusions with the arsenic and cadmium resistance operons of *Staphylococcus aureus* plasmid pI258. FEMS Microbiol. Lett. 110:231–238.

9. Duda, S., and B. Markert. 1992. Baseline concentrations of As, Ba, Be, Li, Nb, Sr and V in surface soils of Poland. Sci. Total Environ. 122:297–290.

10. Frey, J., R. Meier, D. Gygi, and J. Nicolet. 1991. Nucleotide sequence of the hemolysin I gene from *Actinobacillus pleuropneumoniae*. Infect. Immun. 59:3026–3032.

11. Greer, S., and R. N. Perham. 1986. Glutathione reductase from *Escherichia coli*: cloning and sequence analysis of the gene and relationship to other flavoprotein disulfide oxidoreductases. Biochemistry 25:5455–5460.

12. Guzzo, A., C. Diorio, and M. S. DuBow. 1991. Transmission of the *Escherichia coli* Bic gene is regulated by metal ions. Appl. Environ. Microbiol. 57:2255–2259.

13. Guzzo, A., and M. S. DuBow. 1991. Construction of stable, single-copy luciferase gene fusions in *E. coli*. Arch. Microbiol. 156:444–448.

14. Guzzo, A., and M. S. DuBow. 1994. A *luxAB* transcriptional fusion to the *cryptic* *cel* gene of *E. coli* displays increased luminescence in the presence of silver. Mol. Gen. Genet. 242:455–460.

15. Huang, H., C. F. Huang, D. R. Wu, C. M. Jinn, and K. Y. Jan. 1992. Reduction of arsenite to arsenic by the *ars* operon from *Pseudomonas aeruginosa*. Environ. Toxicol. Chem. 11:187–193.

16. Huang, H., C. F. Huang, D. R. Wu, C. M. Jinn, and K. Y. Jan. 1993. Glutathione as a cellular defence against arsenite toxicity in cultured Chinese hamster ovary cells. Toxicology 79:195–201.

17. Huckle, J. W., A. P. Morby, V. Stuehr, and N. J. Robinson. 1993. Isolation of a prokaryotic metallothionein locus and analysis of transcriptional control by trace metal ions. Mol. Biol. 71:177–187.

18. Ji, G., and S. Silver. 1992. Regulation and expression of the arsenic resistance operon from *Staphylococcus aureus* plasmid pI258. J. Bacteriol. 174:3684–3694.

19. Ji, G., and S. Silver. 1992. Reduction of arsenite to arsenate by the *Arc* protein of the arsenic resistance operon of *Staphylococcus aureus* plasmid pI258. Proc. Natl. Acad. Sci. USA 89:9474–9478.

20. Kaneko, M., A. Yamaguchi, and T. Sawai. 1985. Energetics of tetracycline efflux system encoded by Tn10 in *E. coli*. FEBS Lett. 193:194–198.

21. Karkaria, C. E., R. F. Steiner, and B. P. Rosen. 1991. Ligand interactions in the *Arc* protein, the catalytic component of an anion-transporting adenosine-triphosphatase. Biochemistry 30:2625–2628.

22. Kaur, P., and B. P. Rosen. 1992. Plasmid-encoded resistance to arsenic and antimony. Plasmid 27:29–40.

23. Kobayashi, Y., A. Ikiyama, and K. Isono. 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell 50:495–508.

24. Lee, C.-K., and D. A. Cooksey. 1993. Characterization of chromosomal homologs of the plasmid-borne copper resistance operon of *Pseudomonas syringae*. J. Bacteriol. 175:4492–4498.

25. Lindsey, D. M., and J. G. Sanders. 1990. Arsenic uptake and transfer in a simplified estuarine food chain. Environ. Toxicol. Chem. 9:391–395.

26. Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

27. Nordmann, P., and T. Naas. 1994. Sequence analysis of PER-1 extended-spectrum β-lactamase from *Providencia aeruginosa* and comparison with class A β-lactamases. Antimicrob. Agents Chemother. 38:104–114.
27. Ralston, D. M., and T. V. O’Halloran. 1990. Ultrasensitivity and heavy-metal selectivity of the allosterically modulated MerR transcription complex. Proc. Natl. Acad. Sci. USA 87:3846–3850.
28. Roninson, I. B. 1992. From amplification to function: the case of the MDR1 gene. Mutat. Res. 276:151–161.
29. Rosen, B. P., U. Weigel, C. Karkaria, and P. Gangola. 1988. Molecular characterization of an anion pump. J. Biol. Chem. 263:3067–3070.
30. Rosenstein, R., A. Peschel, B. Wieland, and F. Götz. 1992. Expression and regulation of the antimonite, arsenite, and arsenate resistance operon of Staphylococcus xylosus plasmid pSX267. J. Bacteriol. 174:3676–3683.
31. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
32. 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.
33. Seoane, A., and J. M. García Lobo. 1991. Nucleotide sequence of a new class A β-lactamase gene from the chromosome of Yersinia enterocolitica: implications for the evolution of class A β-lactamase. Mol. Gen. Genet. 228:215–220.
34. Silver, S., K. Budd, K. M. Leahy, W. V. Shaw, D. Hammond, R. P. Novick, G. R. Willsky, M. H. Malamy, and H. Rosenberg. 1981. Inducible plasmid-determined resistance to arsenate, arsenite, and antimony(III) in Escherichia coli and Staphylococcus aureus. J. Bacteriol. 146:983–996.
35. Silver, S., G. Nuñofora, L. Chu, and T. K. Misra. 1989. Bacterial resistance ATPases: primary pumps for exporting toxic cations and anions. Trends Biochem. Sci. 14:76–80.
36. Silver, S., G. Nuñofora, and L. T. Phung. 1993. Human Menkes X-chromosome disease and the staphylococcal cadmium-resistance ATPase: a remarkable similarity in protein sequences. Mol. Microbiol. 10:17–12.
37. Silver, S., and M. Walderhaug. 1992. Gene regulation of plasmid- and chromosome-determined inorganic ion transport in bacteria. Microbiol. Rev. 56:195–228.
38. Smith, A. H., C. Hopenhayn-Rich, M. N. Bates, H. M. Goeden, I. Hertz-Picciotto, H. M. Duggan, R. Wood, M. J. Kosnett, and M. T. Smith. 1992. Cancer risks from arsenic in drinking water. Environ. Health Perspect. 97:259–267.
39. Sofia, H. J., V. Burland, D. L. Daniels, G. Plunkett III, and F. R. Blattner. 1994. Analysis of the Escherichia coli genome. V. DNA sequence of the region from 76.0 to 81.5 minutes. Nucleic Acids Res. 22:2576–2586.
40. Sol, K., M. Lapointe, M. MacLeod, C. Nadeau, and M. S. DuBow. 1986. A cloned fragment of HeLa DNA containing consensus sequences of satellite II and III DNA hybridizes with the Drosophila P-element and with the 1.8kb family of human Kpn1 fragments. Biochim. Biophys. Acta 868:128–135.
41. Tisa, L. S., and B. P. Rosen. 1990. Molecular characterization of an anion pump. J. Biol. Chem. 265:190–194.
42. Tolias, P. P., and M. S. DuBow. 1985. The cloning and characterization of the bacteriophage D108 regulatory DNA-binding protein Ner. EMBO J. 4:8031–8037.
43. Vieira, J., and J. Messing. 1987. Production of single-stranded plasmid DNA. Methods Enzymol. 153:8–11.
44. Weis, J. S., and P. Weis. 1992. Construction materials in estuaries: reduction in the epibiotic community on chromated copper arsenate (CCA) treated wood. Mar. Ecol. Prog. Ser. 83:45–53.
45. Wu, J., and B. P. Rosen. 1993. The ArsR protein is a trans-acting regulatory protein. Mol. Microbiol. 8:1331–1336.
46. Wu, J., and B. P. Rosen. 1993. Metalloregulated expression of the ars operon. J. Bacteriol. 175:56–58.
47. Wu, J., and B. P. Rosen. 1993. The arsD gene encodes a second trans-acting regulatory protein of the plasmid-encoded arsenical resistance operon. Mol. Microbiol. 8:615–623.
48. Wu, J., L. S. Tisa, and B. P. Rosen. 1992. Membrane topology of the ArsB protein, the membrane subunit of an anion-translocating ATPase. J. Biol. Chem. 267:12570–12576.