J. Microbiol. Biotechnol. (2004), **14**(3), 593–598

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

© The Korean Society for Microbiology and Biotechnology

Characterization of the Plasmid-Encoded Arsenic Salts Resistance Determinant from *Klebsiella oxytoca* D12

RHIE, HO-GUN, SUNG-JAE LEE, AND HO-SA LEE*

Department of Biology, Research Institute for Basic Science, Kyung Hee University, Seoul 130-701, Korea

Received: November 10, 2003
Accepted: March 25, 2004

Abstract The arsenical resistance (*ars*) operon was cloned from a 67-kilobase pair (kb) plasmid, which was previously shown to be responsible for arsenic salts resistance in *K. oxytoca* D12. When plasmid pAE48, carrying the *ars* operon, was transformed into *E. coli*, transformed cells displayed enhanced survival in the presence of 4 mM arsenite, 50 mM arsenate, or 0.4 mM antimonite. The nucleotide sequence of the 5.6-kb fragment encoding arsenical resistance revealed five open reading frames (ORFs), which were predicted to encode polypeptides of 12.8 (*arsR*), 13.4 (*arsD*), 62.6 (*arsA*), 45 (*arsB*), and 16.7 (*arsC*) kilodaltons (kDa). Each ORF was preceded by a ribosome binding site. A putative promoter-like sequence was identified upstream of *arsR*, and a possible termination site was found downstream of *arsC*. When the deduced amino acid sequences of the *K. oxytoca* D12 Ars proteins were compared with the amino acid sequences of the *E. coli* R773 Ars proteins, a significant amino acid similarity was observed (87.9% for ArsR, 89.2% for ArsD, 83.2% for ArsA, 92.6% for ArsB, and 91.3% for ArsC), suggesting an evolutionary relationship of the *ars* genes of *E. coli* plasmid R773 and *K. oxytoca* D12.

Key words: *Klebsiella oxytoca* D12, plasmid pMH12, *ars* operon

Plasmid-mediated resistance to arsenate, arsenite, and antimonite has been described in both Gram-positive and Gram-negative organisms. Resistance results from the action of an inducible operon-encoded ATP-dependent efflux system that extrudes the toxic oxyanions from the cell, thereby lowering the intracellular concentration of the toxic compounds [4, 23, 25, 33, 34].

The *ars* operon of the *E. coli* plasmid R773 [3, 15] and R46 [2] consists of five genes, *arsR*, *arsD*, *arsA*, *arsB*, and *ars*C. The ArsR protein is a cytoplasmic polypeptide that binds as a dimer to an operator region and represses the functioning of the *ars* operon, including its own synthesis [19, 20, 29]. The ArsD protein is a cytoplasmic secondary regulator, which has a weak affinity to the promoter and is a trans-acting negative repressor [30]. Unlike the ArsR repressor protein, the down-regulation produced by the ArsD is inducer-independent, and its expression has little effect on the level of resistance set by the ArsR [27]. ArsA and ArsB are necessary and sufficient for an ATP-coupled oxyanion pump, which catalyzes extrusion of arsenite and antimonite, producing resistance to these toxic anions [3, 15]. The ArsA is the catalytic subunit of the oxyanion-translocating ATPase and is stimulated by arsenite and antimonate. It is peripherally associated with the cytoplasmic surface of the inner membrane through interaction with the ArsB protein [26]. The ArsA functions as a homodimer on binding of the anions, which is required for hydrolysis of ATP [9]. The ArsB is an inner membrane protein and serves as the membrane anchor for the catalytic ArsA component [26], and mediates electrochemical energy-dependent arsenite efflux in the absence of the ArsA protein, while the ArsA-ArsB complex catalyzes ATP-dependent transport [8, 17, 28]. The ArsC is a soluble polypeptide and functions as arsenate reductase, which converts arsenate to arsenite [10], and is required in addition to ArsA and ArsB to alter the substrate specificity of the pump and transport of the arsenate [17].

The *ars* operon of the *Staphylococcus aureus* plasmid pI258 consists of only three genes, *arsR*, *arsB*, and *ars*C, and endows the cell with resistance to arsenate, arsenite, and antimonite [1, 11, 12]. The ArsR, ArsB, and ArsC function as an inducer-dependent negative repressor of transcription, an inner membrane protein of the arsenite extrusion pump, and an arsenate reductase that converts arsenate to arsenite, respectively [23].

We describe herein the complete nucleotide sequence of the 5.6-kb *Eco*RI fragment of the recombinant plasmid

*Corresponding author
Phone: 82-2-961-0249; Fax: 82-2-964-1131;
E-mail: leehosa@khu.ac.kr

pAE48 which encodes arsenical resistance. The operon encoded ORFs for five polypeptides with molecular sizes of 12.8, 13.4, 62.6, 45, and 16.7 kilodaltons (kDa). The properties of the predicted translation products exhibited a high degree of homology at the amino acid level with those of the ArsR, ArsD, ArsA, ArsB, and ArsC in *E. coli* R773.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Growth Conditions

Table 1 lists the genotypes and origins of strains of *E. coli* and plasmids which were used in this study. Cells were grown in Luria Bertani (LB) medium at 37°C [18], unless otherwise noted. Ampicillin (100 µg/ml), kanamycin (50 µg/ml), rifampicin (200 µg/ml), and tetracycline (15 µg/ml), and isopropyl-β-D-thiogalactopyranoside (IPTG) were added as required.

For assays for resistance to arsenate, arsenite, and antimonite, overnight culture in LB was diluted 100-fold in LB containing various concentrations of arsenic salt. The cultures were then incubated for 12 h at 37°C with shaking, and the optical density at 600 nm was measured.

Genetic Techniques

Standard molecular genetic techniques were performed as described by Sambrook *et al.* [18] or as per the manufacturer's instruction manual unless mentioned specially. DNA sequences were determined by the dideoxy-mediated termination method of Sanger *et al.* using an ABISEQ automatic sequencer. Nucleotide and amino acid sequences were analyzed with the sequence analysis software DNAMAN (Lynnon Biosoft), Clone Manager 5 (Scientific & Educational software), and Align Plus (Scientific & Educational software). Homology searches were carried out by the BLAST search algorithms located at National Center for Biotechnology Information site (http://www.ncbi.nlm.nih.gov/) on the World Wide Web.

Protein Expression

The *ars* genes were expressed by the T7 RNA polymerase-promoter system [24]. The genes were cloned into a T7 promoter-containing vector pT7-7, and the resulting plasmid was transformed into *E. coli* BL21 (DE3) bearing the T7 RNA polymerase gene for expression of the target protein. Plasmid-bearing cells were grown overnight at 37°C in LB medium containing ampicillin (50 µg/ml). The culture was diluted 100-fold into M9 medium containing amino acids except methionine and cysteine. When the culture reached the mid-log phase, 1.0 mM IPTG and 50 µCi [^{35}S]-methionine were added to induce gene expression as well as to label proteins. Labeled cells were solubilized and analyzed by 12% SDS-PAGE. Gels were fixed, stained with Coomassie blue, dried onto filter paper, and autoradiographed.

Nucleotide Sequence Accession Number

The nucleotide sequence of the *ars* operon has been submitted to GenBank and assigned the accession no. AF168737.

RESULTS

Cloning and Expression of the *ars* Operon

A 5.6-kb *Eco*RI DNA fragment was cloned in several steps from the 67-kb cryptic plasmid pMH12 of *Klebsiella*

Table 1. Bacterial strains and plasmids used in this study.

Strains or plasmids		Relevant characteristics	Reference or source
Strains			
E. coli			
	DH5α	*Sup*E44 Δ*lac*U169 (φ80 *lacZ* ΔM15) *hsd*R17 *rec*A1 *end*A1 *rel*A1	GIBCO BRL (USA)
	BL21(DE3)	F *dcm ompT hsdS* ($_{rB-mb}^{-}$) *galDE3*	Tabor *et al.* [24]
Plasmids			
	pUC18	Cloning vector, Ap	TaKaRa (Japan)
	pT7-5, 7	Expression vector	Tabor *et al.* [24]
	pMH12	67-kb plasmid from the *K. oxytoca* D12	Chung *et al.* [6]
	pAE48	5.6-kb *Eco*RI fragment of pMH12 cloned into pUC18; *ars*RDABC	Chung *et al.* [6]
	pAE65	pUC18, 4.2 kb *Nde*I-*Eco*RI	Chung *et al.* [6]
	pAE67	pUC18, 4.2 kb *Eco*RI-*Acc*I	Chung *et al.* [6]
	pAE70	pUC18, 3.0 kb *Bgl*II-*Acc*I	This study
	pFT17	1.7-kb *Bgl*II fragment into pT7-7	This study
	pFT25	2.5-kb *Nde*I-*Xho*I fragment into pT7-7	This study
	pFT27	2.7-kb *Nde*I-*Acc*I fragment into pT7-7	This study
	pFT56	5.6-kb *Eco*RI fragment into pT7-7	This study
	pJM56	5.6-kb *Eco*RI fragment into pT7-5	This study

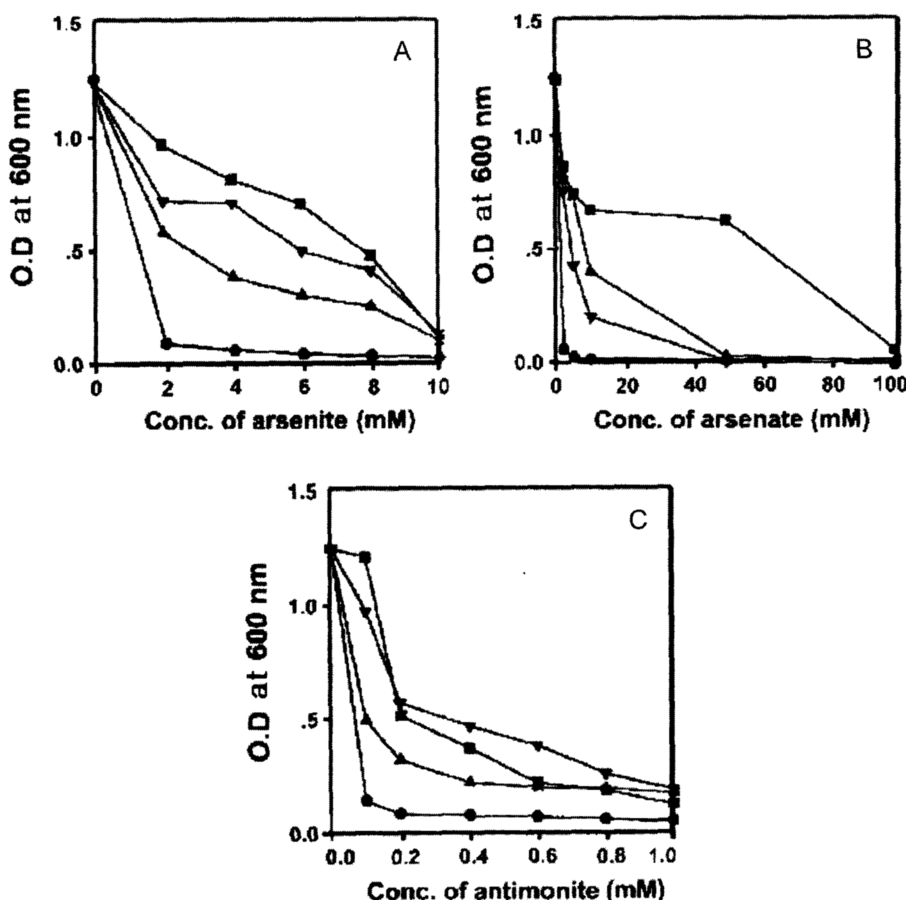

Fig. 1. Resistance to arsenite, arsenate, and antimonite mediated by cloned *ars* genes of *K. oxytoca* D12.
Resistance to arsenite (A), arsenate (B), and antimonite (C) was examined in *E. coli* DH5α with vector only (●), pAE48 (*arsRDABC*) (■), pAE65 (*arsBC*) (▲), or pAE67 (*arsRDA*) (▼). The overnight culture in LB was diluted 100-fold in LB and the cultures were then incubated for 12 h at 37°C with shaking, and the optical density at 600 nm was measured.

oxytoca D12 into the unique *Eco*RI site of vector pUC18, forming pAE48 [5, 6]. When plasmid pAE48 was transformed into the *E. coli*, transformant cells grew in the presence of 4 mM arsenite, 50 mM arsenate, or 0.4 mM antimonite. However, cells without pAE48 failed to grow in the presence of 2 mM arsenate, 5 mM arsenate, or 0.1 mM antimonite (Fig. 1). These results indicate that the 5.6-kb *Eco*RI fragment contains the intact determinant of arsenic resistance and functions in *E. coli*.

Nucleotide Sequence of the *ars* Operon

The complete nucleotide sequences of the cloned fragment were determined in both strands. In the 5.6-kb *Eco*RI region, five potential ORFs for protein-coding regions were identified by computer analysis along with the proposed initiation and stop codons, the proposed ribosome-binding site, and the deduced amino acid sequences of the five protein-coding regions. The amino acid sequence revealed highest homology to ArsR, ArsD, ArsA, ArsB, and ArsC in *E. coli* plasmid R773 among those in other Gram-negative and Gram-positive bacteria (data not shown). The *ars* locus of *Klebsiella oxytoca* consisted of a *arsABC* operon, which was the same in organization from the corresponding operon in *E. coli* plasmid R773. The five ORFs were oriented in the same direction and followed 20-bp downstream from the stop codon of *arsC* by a 21-bp palindromic sequence. This palindrome, corresponding to an mRNA hairpin structure with a ΔG of -23.2 kcal/mol, may function as a transcription terminator. Each ORF contained an AUG translation start codon together with a properly spaced ribosome binding sequence.

In the region upstream of *arsA*, two ORFs (348 and 360 bp) were oriented in the same direction as that of the other three genes. The first ORF encoded proteins composed of 116 amino acids, and the deduced amino acid sequence was similar to the ArsR of *E. coli* R773 (87.9% identity), which functions primarily in a negatively acting repressor protein [27]. The DNA-binding helix-turn-helix region and metal-binding motif are highly conserved in all known ArsR (Fig. 2), therefore, the ORF was referred to as

ArsR R773	1	MLQLTPLQLF KNLSDETRLG IVLLLREMG	29
ArsR pMH12	1	MK. LTTLQLF KYLSDETRLG IVLLLREMG	28
ArsR R773	30	ELCVCDLCMALDQSQPKISRHLAMLRESGI LL	60
ArsR pMH12	29	ELSVCDLC TALEQ SQPKISRHLAMLRESGLLL	60

Helix-Turn-Helix

Fig. 2. Alignment of the ArsR proteins of *E. coli* plasmid R773 and *K. oxytoca* D12 plasmid pMH12.
Amino acid sequences which are conserved in six of the seven sequences are highlighted. The location of the putative helix-turn-helix DNA-binding motif is indicated by a dashed line.

arsR. The second ORF with 120 amino acids was also identified downstream of *arsR*, and the deduced amino acid sequences exhibited great identity to the *arsD* of *E. coli* R773 (89.2%). Accordingly, the ORF was designated as *arsD*. The nucleotide sequence resembling the -35 to -10 consensus sequence of the σ^{70}-dependent promoter and the imperfect inverted repeat of a putative operator were found upstream of *arsR*, although their relevance has not yet been explored.

The *arsA* encoded protein of 585 amino acids was 83.2% identical with the sequences of R773. ArsA consisted of two independent domains with 32% homology and was connected by a short linker sequence. Each domain contained canonical ATP-binding motif GKGGVGKTS, which is required for ATP-dependent efflux activity and resistance.

The *arsB* revealed homologies to gene encoding ArsB in *E. coli* R773 and encoded protein composed of 429 amino acids (92.6% identity). With 62.2% nonpolar residues, the protein would be expected to be hydrophobic. The hydropathy profile demonstrates the hydrophobic character of the ArsB protein, and it has been proposed to form a transmembrane channel to pump AsO_2-across the membrane (data not shown) [28].

The *arsC* is the smallest structural gene of the *ars* operon and encoded protein with 137 amino acids. The deduced amino acid sequences exhibited greater identity to the ArsC of *E. coli* R773 (91.3%) than to the staphylococcal ArsC protein (22%).

Expression of *ars* Operon Genes in *E. coli*

The four *ars* proteins were identified in *E. coli* BL21 (DE3) after cloning into an expression vector, in which the *ars* operon was placed under the control of the T7 promoter. The *ars* fragments inserted into the pT7-7 vector are shown in Fig. 4, and they were transferred into *E. coli*. Cells harboring the pT7-7 with *ars* fragments were cultivated in the presence of sodium arsenite, and the total proteins were analyzed by 12% acrylamide gel. Plasmid-encoded synthesis of [^{35}S]-methionine-labeled protein is shown in Fig. 3. When the intact *ars* operon was inserted into the pT7-7, which yielded pFT56, four polypeptides were identified (Fig. 3A, lane 5) with apparent molecular massess of 12.8 kDa, 13.4 kDa, 16.7 kDa, and 62.6 kDa, as predicted for ArsR, ArsD, ArsC, and ArsA, respectively. That the 14.7-kDa protein was synthesized in this strain may reflect either degradation of 62.6 kDa or the expression of the gene in the upstream of the promoter region. The opposite direction clone examined, that in pJM56, eliminated synthesis of those proteins and resulted in a fragment of 20.5 kDa product (Fig. 3A, lane 4). This could have been derived from a gene that could transcribe in the orientation opposite to the *ars* operon. Therefore, these results confirm the direction of transcription of the operon. The putative ArsB with an apparent molecular mass of 45 kDa was not expressed with the T7-7 promoter system, which might have been due to its location in the cytoplasmic membrane. With plasmid pFT17 containing *arsD* and partially deleted *arsR*, the ArsD polypeptide was produced, and the size of the polypeptide attributed to ArsR was reduced (Fig. 3B, lane 3). The total deletion of *arsRDC* resulted in a ArsA and smaller bands that might be degradation products of ArsA or the results of incorrect start or stop points (Fig. 3B, lanes 1 and 2).

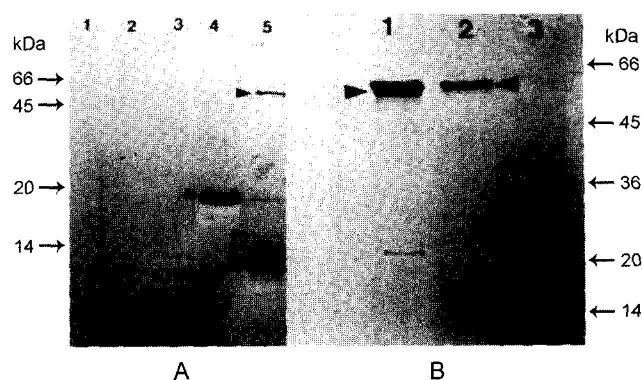

Fig. 3. Expression of the *K. oxytoca* D12 *ars* proteins in *E. coli* under control of the bacteriophage T7 promoter.
Plasmid constructions used are listed in Table 1. The experimental procedure is described in Materials and Methods. (A) Lane 1, marker protein. Lane 2, plasmid pT7-5 without insert. Lane 3, plasmid pT7-7 without insert. Lane 4, pJM56 with intact *ars* determinants in the orientation opposite to the T7 promoter. Lane 5, pFT56 with intact *ars* determinants. (B) Lane 1, pFT25 with *arsA* (total) and *arsB* (partial). Lane 2, pFT27 with *arsA* (total) and *arsB* (partial). Lane 3, pFT17 with *arsRD* (total) and *arsA* (partial). The positions of approximate molecular weight of marker positions are noted on the left and right.

DISCUSSION

The members of the ArsR family of repressor were postulated to have a metal-binding domain, followed by a DNA-binding domain [31, 32]. Metal-binding sites conserved as "ELCVCDL" and DNA-binding sites conserved as a "helix-turn-helix motif" are predicted for a metal-dependent repressor [21, 22]. Analysis of the nucleotide sequences of the *arsR* gene from *K. oxytoca* D12 demonstrated that the sequence "ELSVCDL" was located just outside of the

helix-turn-helix region, which started at residue 35. In ArsR of R773, the pair of Cys-32 and Cys-34 is conserved and an important component of the metal recognition site. However, there was only one Cys-33 in the putative metal-binding domain of ArsR from *K. oxytoca*. Therefore, we presumed that Ser-31 and Cys-33 could be the ligands to arsenate, which result in induction of conformational change in the helix-turn-helix domain and dissociation of the repressor-DNA complex. The product of *arsD* was 89% identical to ArsD protein of R773, and may play a role as secondary regulatory protein. The ArsA has been shown to undergo a specific conformation change upon ATP binding [13]. From the analysis of the predicted amino acid sequence, the ArsA protein appeared to have two nucleotide binding sites with a characteristic Walker A sequence [13]. Both the N-terminal and C-terminal contained the glycine-rich clusters, $G_{15}KGGVGKTS_{23}$ and $G_{335}KRCVGKT_{343}$, suggesting that the ArsA protein is the catalytic subunit of an arsenate-translocating ATPase [4]. The predicted ArsA protein also contained two independent domains with 32% homology, possibly due to gene duplication and fusion of a gene ancestral to the *arsA* gene [4]. It was also shown that ArsA was 32% identical to the sequence of MinD, which is a membrane-associated protein capable of binding and hydrolyzing ATP [7]. MinD functions to activate the division inhibition activity of MinC, presumably by mediating the membrane attachment of MinC [16]. We demonstrated that an overexpression of *arsA* in *E. coli* leads to an inhibition of septation at all potential division sites, resulting in the formation of long nonseptate filaments [14]. The ArsB protein is a hydrophobic protein and likely a membrane-associated protein. The hydropathy profile indicated that the *K. oxytoca* D12 ArsB protein had 12 membrane-spanning regions, suggesting that the ArsB protein might have a function similar to that of other ArsB proteins [28].

The *arsRDABC* genes in *K. oxytoca* D12 seem to be organized as an operon. The conclusion is based on (i) the identification of a single potential promoter not only of *arsR,* but also of the complete operon, (ii) the location of a potential transcription terminator detected downstream from *arsC,* and (iii) the only predicted ribosome site upstream of *arsD*, *arsA*, *arsB*, and *arsC*.

More detailed analysis is necessary to define the progenitor of the plasmid-borne *ars* operon and the possibility of lateral gene transfer for the *ars* operon between *E. coli* and *K. oxytoca*.

Acknowledgments

This research was supported by the Kyung Hee University Research Fund in 1999 (KHU-1U0199009) and Korea Research Foundation in 1999 (015-D00216).

REFERENCES

1. Bröer, S., J. I. Guangyong, A. Bröer, and S. Silver. 1993. Arsenic efflux governed by the arsenic resistance determinant of *Staphylococcus aureus* plasmid pI258. *J. Bacteriol.* **175:** 3480-3485.
2. Bruhn, D. F., J. Li, S. Silver, F. Roberto, and B. P. Rosen. 1996. The arsenical resistance operon of IncN plasmid R46. *FEMS Microbiol. Lett.* **139:** 149-153.
3. Chen, C., H. L. Mobley, and B. P. Rosen. 1985. Separate resistances to arsenate and arsenite (antimonate) encoded by the arsenical resistance operon of R factor R773. *J. Bacteriol.* **161:** 758-763.
4. Chen, C. M., T. K. Misra, S. Silver, and B. P. Rosen. 1986. Nucleotide sequence of the structural genes for an anion pump. *J. Biol. Chem.* **261:** 15030-15038.
5. Chung, M. K. and H. S. Lee. 1991. Genetic characteristics of arsenic compounds-resistant bacteria isolated from stream water. *Kor. J. Microbiol.* **29:** 63-68.
6. Chung, M. K., N. J. Chung, and H. S. Lee. 1993. Construction of a cloning vector carrying arsenic compound resistant genes. *Kor. J. Microbiol.* **31:** 404-410.
7. de Boer, P. A. J., R. E. Crossley, A. R. Hand, and L. I. Rothfield. 1991. The MinD protein is a membrane ATPase required for the correct placement of the *Escherichia coli* division site. *EMBO J.* **10:** 4371-4380.
8. Dey, S. and B. P. Rosen. 1995. Dual mode of energy coupling by the oxyanion-translocating ArsB protein. *J. Bacteriol.* **177:** 385-389.
9. Hsu, C. M., P. Kaur, C. E. Karkaria, R. F. Steiner, and B. P. Rosen. 1991. Substrate-induced dimerization of the ArsA protein, the catalytic component of an anion-translocating ATPase. *J. Biol. Chem.* **266:** 2327-2332.
10. Ji, G. and S. Silver. 1992. Reduction of arsenate to arsenite by the ArsC protein of the arsenic resistance operon of *Staphylococcus aureus* plasmid pI258. *Proc. Natl. Acad. Sci. USA* **89:** 9474-9478.
11. Ji, G. and S. Silver. 1992. Regulation and expression of the arsenic resistance operon from *Staphylococcus aureus* plasmid pI258. *J. Bacteriol.* **174:** 3684-3694.
12. Ji, G., E. A. Garber, L. G. Armes, C. M. Chen, J. A. Fuchs, and S. Silver. 1994. Arsenate reductase of *Staphylococcus aureus* plasmid pI258. *Biochemistry* **33:** 7294-7299.
13. Kaur, P. and B. P. Rosen. 1994. Identification of the site of [α-^{32}P]ATP adduct formation in the ArsA protein. *Biochemistry* **33:** 6456-6461.
14. Lee, S. J., S. C. Lee, S. H. Choi, M. K. Chung, H. G. Rhie, and H. S. Lee. 2001. Effect of ArsA, arsenite-specific ATPase, on inhibition of cell division in *Escherichia coli. J. Microbiol. Biotechnol.* **11:** 825-830.
15. Mobley, H. L., T. C. Chen, S. Silver, and B. P. Rosen. 1983. Cloning and expression of R-factor mediated arsenate resistance in *Escherichia coli. Mol. Gen. Genet.* **191:** 421-426.
16. Raskin, D. M. and P. A. J. de Boer. 1999. MinDE-dependent pole-to-pole oscillation of division inhibitor MinC in *Escherichia coli. J. Bacteriol.* **181:** 6419-6424.

17. Rosen, B. P., U. Weigel, R. A. Monticello, and B. P. F. Edwards. 1991. Molecular analysis of an anion pump: purification of the ArsC protein. *Arch. Biochem. Biophys.* **284:** 381-385.
18. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning; A Laboratory Manual*, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, U.S.A.
19. San Francisco, M. J. D., L. S. Tisa, and B. P. Rosen. 1989. Identification of the membrane component of the anion pump encoded by the arsenical resistance operon of plasmid R773. *Mol. Microbiol.* **3:** 15-21.
20. 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.
21. Shi, W., J. Wu, and B. P. Rosen. 1994. Identification of putative metal binding site in a new family of metalloregulatory proteins. *J. Biol. Chem.* **269:** 19826-19829.
22. Shi, W., J. Dong, R. A. Scott, M. Y. Kzenzenko, and B. P. Rosen. 1996. The role of arsenic-thiol interactions in metalloregulation of the *ars* operon. *J. Biol. Chem.* **271:** 9291-9297.
23. Silver, S. and T. K. Misra. 1988. Plasmid-mediated heavy metal resistance. *Annu. Rev. Microbiol.* **42:** 717-743.
24. Tabor, S. and C. C. Richardson. 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled expression of specific genes. *Proc. Natl. Acad. Sci. USA* **82:** 1074-1078.
25. Tisa, L. S. and B. P. Rosen. 1990. Transport system encoded by bacterial plasmids. *J. Bioenerg. Biomembr.* **22:** 493-507.
26. Tisa, L. S. and B. P. Rosen. 1990. Molecular characterization of an anion pump. *J. Biol. Chem.* **265:** 190-194.
27. Wu, J. and B. P. Rosen. 1991. The ArsR protein is a trans-acting regulatory protein. *Mol. Microbiol.* **5:** 1331-1336.
28. 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.
29. Wu, J. and B. P. Rosen. 1993. Metalloregulated expression of the *ars* operon. *J. Biol. Chem.* **268:** 52-58.
30. Wu, J. and B. P. Rosen. 1993. The *ars*D gene encoded a second *trans*-acting regulatory protein of the plasmid-encoded arsenical resistance operon. *Mol. Microbiol.* **8:** 615-623.
31. Xu, C., W. Shi, and B. P. Rosen. 1996. The chromosomal *arsR* gene of *Escherichia coli* encodes a trans-acting metalloregulatory protein. *J. Biol. Chem.* **271:** 2427-2432.
32. Xu, C. and B. P. Rosen. 1997. Dimerization is essential for DNA binding and repression by the ArsR metalloregulatory protein of *Escherichia coli. J. Biol. Chem.* **272:** 15734-15738.
33. Yoon, K. P. 2002. Plasmid-mediated arsenical and antimonial resistance determinants (*ars*) of *Pseudomonas* sp. KM20. *J. Microbiol. Biotechnol.* **12:** 31-38.
34. Yoon, K. P. 2003. Construction and characterization of multiple heavy metal-resistant phenol-degrading *Pseudomonads* strains. *J. Microbiol. Biotechnol.* **13:** 1001-1006.