

# The *ars* Operon of *Escherichia coli* Confers Arsenical and Antimonial Resistance

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**The chromosomally encoded arsenical resistance (*ars*) operon subcloned into a multicopy plasmid was found to confer a moderate level of resistance to arsenite and antimonite in *Escherichia coli*. When the operon was deleted from the chromosome, the cells exhibited hypersensitivity to arsenite, antimonite, and arsenate. Expression of the *ars* genes was inducible by arsenite. By Southern hybridization, the operon was found in all strains of *E. coli* examined but not in *Salmonella typhimurium*, *Pseudomonas aeruginosa*, or *Bacillus subtilis*.**

Resistance to arsenite ( $\text{As}^{3+}$ ), antimonite ( $\text{Sb}^{3+}$ ), and arsenate ( $\text{As}^{5+}$ ) is found in both gram-negative and gram-positive bacteria (17). High-level resistance has been associated with plasmid-encoded arsenical resistance (*ars*) operons (12, 25, 26, 40). These plasmid-encoded metalloid resistances were widespread in bacterial species even before the emergence of resistances to most antibiotics (14). The *ars* operon carried on the *Escherichia coli* R factor R773 (10, 12) encodes a transport system that extrudes arsenate, arsenite, and antimonite; the lowering of the intracellular concentration of toxic oxyanion produces resistance (24, 30, 36). The *ars* operon of the *E. coli* conjugal plasmid R773 has five genes, *arsRDABC* (4, 34, 45). The *arsR* and *arsD* genes encode regulatory proteins (43–45). The *arsA* and *arsB* genes encode the subunits of an ATP-driven arsenite pump (7). The ArsA protein is the catalytic subunit of the pump (13), while the ArsB protein is the membrane sector (8, 42). In the absence of the *arsA* gene, the *arsB* gene product alone provides partial arsenite resistance, most likely by functioning as a secondary uniporter (7a, 9). Arsenate resistance is conferred by reduction to arsenite by the *arsC* gene product; the resulting arsenite is extruded by the transport system (11, 27).

The staphylococcal plasmids pI258 and pSX267 also carry *ars* operons (15, 31). Those operons have only three genes, *arsRBC*, lacking *arsD* and *arsA* genes. Resistance also results from active extrusion of arsenite, and the gram-positive homologs of the ArsB protein have also been proposed to function as secondary porters (3). Although the R773 and pI258 ArsB proteins are only 58% similar in sequence, secondary structural predictions suggest that the two proteins are topologically much more similar, and functional chimeras of the two have been constructed, suggesting that the two ArsB proteins function similarly at the biochemical level (9). Similarly, the ArsC protein of plasmid pI258 is also an arsenate reductase (16), even though there is little sequence similarity between the ArsC proteins of plasmids R773 and pI258. The ArsR proteins of the plasmids of the gram-positive and gram-negative organisms exhibit only 30% sequence similarity, but both are arsenite-responsive repressor proteins (31, 43).

Recently Sofia and coworkers (39) determined the sequence of a 225.4-kb segment of the *E. coli* genome corresponding to min 76.0 to 81.5 on the genetic map. Three open reading

frames, termed *arsE*, *arsF*, and *arsG*, were identified as homologs of the plasmid R773 *arsR*, *arsB*, and *arsC* genes. Since the genes and proteins of three other *ars* operons have been designated R, B, and C, we refer in this communication to the *arsE*, *arsF*, and *arsG* genes as the *E. coli* chromosomal *arsR*, *arsB*, and *arsC* genes and their gene products as ArsR, ArsB, and ArsC proteins.

Plasmidless strains of *E. coli* are intrinsically more resistant to arsenite and antimonite than are plasmidless strains of *Staphylococcus aureus*, requiring 5- to 10-times-higher concentrations to produce the same degree of growth inhibition (36). To examine whether the chromosomal *arsRBC* operon is responsible for this basal level of arsenical resistance, the *arsRBC* genes were cloned onto a high-copy-number plasmid. Expression from the plasmid conferred increased arsenite resistance. Proteins that reacted with antibodies against the ArsR and ArsC proteins were produced; presumably the cross-reacting species are the products of the chromosomal *arsR* and *arsC* genes. Expression of the cross-reacting proteins was arsenite inducible, consistent with the chromosomally encoded ArsR protein being a functional homolog of the R773 ArsR repressor. The majority of the *ars* sequence was replaced on a plasmid by a chloramphenicol resistance cassette, and the chromosomal operon was replaced with the operon deletion by homologous recombination. The resulting  $\Delta$ *arsRBC* strain was hypersensitive to arsenite. Transformation of the deletion strain with high-copy-number plasmids carrying either the chromosomal *arsRBC* or R773 *arsRBC* genes conferred arsenite resistance. Thus, the physiological role of the chromosomal *ars* operon appears to be to confer low-level arsenical resistance.

## MATERIALS AND METHODS

**Bacterial strains, plasmids, and bacteriophage.** The bacterial strains, plasmids, and bacteriophage used in this study are described in Table 1.

**Media and growth conditions.** Cells were grown at 37°C in LB or M9 medium (33) unless otherwise noted. Ampicillin (100 µg/ml), chloramphenicol (20 µg/ml), tetracycline (15 µg/ml), isopropyl-β-D-thiogalactopyranoside (IPTG; 0.1 mM), and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal; 80 µg/ml) were added as required.

**Protein electrophoresis and immunoblotting.** Cultures of cells were grown in 1 ml of LB medium for 4 to 6 h with or without induction, as described. The cells were pelleted by centrifugation and suspended in 0.1 ml of sodium dodecyl sulfate (SDS) sample buffer. After boiling for 10 min, whole-cell proteins were separated by SDS-polyacrylamide gel electrophoresis (PAGE) on 12 to 15% acrylamide gels as described by Laemmli (19), with subsequent immunoblotting using antiserum against the R773 ArsR or ArsC protein. Immunoblotting was performed by using an enhanced chemiluminescence assay (Amersham) and exposure on X-ray film at room temperature as described previously (7).

**Resistance assays.** For assays of resistance to arsenite or antimonite in liquid

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TABLE 1. Strains, phage, and plasmids used

| Strain, phage, or plasmid              | Genotype and phenotype  | Reference or source   |
|--|---|-----------------------|
| <b>Strains</b>                         |   |                       |
| <i>Escherichia coli</i>                |   |                       |
| W3110                                  | K12 F <sup>-</sup> IN( <i>rrnD-rrnE</i> )   | 2                     |
| AW3110                                 | W3110 $\Delta$ ars::cam   | This study            |
| JM109                                  | <i>recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi</i> $\Delta$ ( <i>lac-proAB</i> ) F'<br>[ <i>traD36 proAB<sup>+</sup> lacI<sup>q</sup> lacZ</i> $\Delta$ M15] | 33                    |
| HB101                                  | <i>supE44 hsdS20</i> (r <sub>B</sub> <sup>-</sup> m <sub>B</sub> <sup>-</sup> ) <i>recA3 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1</i>                    | 33                    |
| LE392 $\Delta$ uncIC                   | <i>supE44 supF58 hsdR514 galK2 galT22 metB1 trpR55 lacY1</i> $\Delta$ uncIC   | 1                     |
| LE392 $\Delta$ uncIC $\Delta$ ars::cam | P1 transduction of $\Delta$ ars::cam from AW3110 to LE392 $\Delta$ uncIC  | This study            |
| CC118                                  | <i>araD139</i> $\Delta$ ( <i>ara-leu</i> )797 $\Delta$ lacX74 <i>galE galK</i> $\Delta$ phoA20 <i>thi rpsE rpoB argE</i> (Am) <i>recA1</i>                  | 21                    |
| K-12                                   | $\lambda$ <sup>+</sup>  | 2                     |
| K24                                    | Su <sup>o</sup> , $\lambda$ cI857 Nam7,53   | E. Olson, Parke-Davis |
| <i>Bacillus subtilis</i> IS75          | <i>leu met his</i>  | 38                    |
| <i>Pseudomonas aeruginosa</i> PAO      | Wild type   | ATCC 15692            |
| <i>Salmonella typhimurium</i> LT2      | Wild type   | ATCC 14028            |
| <b>Phage</b>                           |   |                       |
| $\lambda$ EC27-334                     | Chromosomal <i>arsRBC</i> genes from <i>E. coli</i> K-12 strain MG1655  | 39                    |
| $\lambda$ G501                         | $\lambda$ <i>imm</i> <sup>434</sup> <i>cIts</i> Nam7,53   | E. Olson, Parke-Davis |
| <b>Plasmids</b>                        |   |                       |
| pBR325                                 | Cloning vector (Cm <sup>r</sup> Tc <sup>r</sup> Ap <sup>r</sup> )   | 29                    |
| pUC19                                  | Cloning vector (Ap <sup>r</sup> )   | 46                    |
| pWSU1                                  | <i>arsRDABC</i> operon from R factor R773 in pBR322   | 34                    |
| pBC101                                 | Removal of a 1,143-bp <i>NdeI-PstI</i> fragment containing the <i>arsA</i> gene from pWSU1, <i>arsRDBC</i>  | This laboratory       |
| pWPS1                                  | Chromosomal <i>ars</i> operon in pUC19  | This study            |
| pWPS1 $\Delta$ ars::cam                | $\Delta$ ars::cam in pWPS1  | This study            |
| pArsA                                  | R773 <i>arsA</i> gene in pACYC184   | This laboratory       |

media, overnight cultures were diluted 100-fold into fresh LB medium containing the appropriate antibiotics and various concentrations of sodium arsenite or potassium antimonic tartrate. The cultures were incubated at 37°C with shaking for 6 to 24 h, and the  $A_{600}$  was determined. For arsenate resistance assays in liquid media, overnight cultures grown in low-phosphate media were diluted 100-fold into fresh low-phosphate media supplied with antibiotics and various concentrations of sodium arsenate (27). The cultures were incubated at 37°C with shaking for 16 h, and the optical density at 600 nm was measured. For resistance assay on solid media, 0.1 ml of late-log-phase cells was spread on a petri dish, and various concentrations of the compounds to be tested were added to sensitivity disks on the surface of the agar. The plates were incubated at 37°C overnight, and the diameters of zones of inhibition were compared.

**Cloning of the chromosomal *arsRBC* operon and construction of strain AW3110 ( $\Delta$ arsRBC::cam).** DNA from the obligate lytic phage  $\lambda$ EC27-334 containing the chromosomal *arsRBC* operon (39) was digested with *HincII*. A mixture of *HincII* fragments of about 3.2 kb, the predicted size of the *ars* operon, was purified and inserted into the *HincII* site of vector plasmid pUC19. The recombinant plasmids were transformed into *E. coli* JM109, and white colonies were selected on LB plates containing ampicillin, IPTG, and X-Gal. White colonies were screened for resistance to 4 mM sodium arsenite. *E. coli* JM109 harboring plasmid pBC101 (*arsRDBC* from R773) was used as a positive control. A plasmid designated pWPS1 was shown by restriction digestion and DNA sequencing to contain the *arsRBC* operon. A derivative of pWPS1 in which a portion of the *ars* operon was replaced with a chloramphenicol resistance gene (pWPS1 $\Delta$ arsRBC::cam) was constructed. A 2-kb *BglIII-EcoRV* fragment containing the entire *arsB* and *arsC* genes and 60% of the 3' portion of the *arsR* gene was removed, and a 1.3-kb *BstUI* fragment containing the *cam* gene from pBR325 (29) was inserted at the same site with blunt-end ligation.

Isolation of AW3110 ( $\Delta$ arsRBC::cam) was carried out by using a modified procedure for allelic replacement (18). The obligate lytic phage  $\lambda$ EC27-334 was crossed with  $\lambda$ G501 ( $\lambda$  *imm*<sup>434</sup> *cIts* Nam7,53) by coinfection of *E. coli* LE392 with each phage at a multiplicity of infection of 5. The resulting lysate was plated on a lawn of *E. coli* K24 (Su<sup>o</sup>,  $\lambda$ cI857 Nam7,53) to select for  $\lambda$ EC27-334 derivatives that contained the *imm*<sup>434</sup> *cIts* allele. Recombinants were screened for the presence of the *arsRBC* operon by plaque hybridization using random-prime-labeled *arsBC* as a probe. A lysate of one recombinant,  $\lambda$ imm<sup>434</sup>-EC27, was made by growing the phage on *E. coli* W3110 carrying pWPS1 $\Delta$ ars::cam to allow for replacement by recombination of the *ars* allele on the phage with the  $\Delta$ ars::cam allele on the plasmid. The resulting lysate was used to infect W3110 with a multiplicity of infection of 0.1, and Cm<sup>r</sup> colonies were isolated at 30°C on LB plates containing 20  $\mu$ g of chloramphenicol per ml. Colonies were screened for ampicillin sensitivity to exclude recombinants that transferred the plasmid

and phage. One colony, designated strain AW3110, was shown to be a stable double recombinant by Cm<sup>r</sup> selection at 42°C. AW3110 was shown by colony hybridization to lack the *ars* genes. The chromosomal  $\Delta$ ars::cam was transferred from strain AW3110 to strain LE392 $\Delta$ uncIC by generalized transduction using P1 bacteriophage as described previously (23), with selection for Cm<sup>r</sup>, and the transfer was confirmed by Southern blotting.

**DNA manipulation and sequence analysis.** Plasmid DNA was prepared by using a Wizard DNA purification system (Promega). Small-scale purification of bacterial chromosomal DNA was done as described by Lewington et al. (20). Bacteriophage  $\lambda$  preparation, restriction endonuclease digestions, Klenow fragment fill-in, and ligations were performed as described elsewhere (33). One-step preparation of competent cells and plasmid transformations were performed by the method of Chung et al. (6). DNA fragments were separated by electrophoresis on 1% agarose gels. The desired fragment was recovered from low-melting-point agarose gels and purified by a Wizard cleanup system (Promega). For DNA sequencing, double-stranded DNA was denatured with 0.2 M NaOH and 0.2 mM Na<sub>2</sub>EDTA for 30 min at 37°C. The sequencing assays (35) were performed with a Sequenase version 2.0 system (U.S. Biochemical).

**Colony, plaque, and Southern hybridizations.** Colony, plaque, and Southern hybridizations were performed by published methods (33). A probe consisting of a 1.2-kb *EcoRV* fragment from pWPS1 containing the 3' 58% of the *arsB* gene and the entire *arsC* gene was randomly labeled with [ $\alpha$ -<sup>32</sup>P]dATP, using a Random Primer DNA Labeling System from Bethesda Research Laboratories according to the manufacturer's directions. For Southern hybridizations, DNA was extracted from cells grown overnight at 37°C in 2 ml of LB medium, digested with *HincII*, and electrophoresed on 0.8% agarose gels. Hybridization was performed by incubation with a labeled probe overnight at 65°C. Labeled bands were visualized by autoradiography.

**Arsenite accumulation in cells.** Cells were grown to late exponential phase at 37°C with aeration in a medium consisting of 50 mM triethanolamine HCl (pH 6.9), 15 mM KCl, 10 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, and 1 mM Mg<sub>2</sub>SO<sub>4</sub> supplemented with 0.5% glycerol, 2.5  $\mu$ g of thiamine per ml, 0.5% peptone, and 0.15% succinate. The cells were harvested, washed, and suspended in 0.5 to 1 ml of the same medium, all at room temperature. To initiate the transport assay, 50  $\mu$ l of cells was diluted into 0.6 ml of the same medium containing 20 mM glucose, 0.1 mM NaAsO<sub>2</sub>, and 1.25  $\mu$ Ci of <sup>73</sup>AsO<sub>2</sub><sup>-</sup>. Samples (0.1 ml) were withdrawn at intervals, filtered through nitrocellulose filters (0.45- $\mu$ m pore diameter; Whatman), and washed with 5 ml of the same medium. The filters were dried and quantified by liquid scintillation counting.

## RESULTS

**Comparison of the gene products of the plasmid and chromosomal *ars* operons.** The predicted amino acid sequences of the ArsR, ArsB, and ArsC proteins of the chromosomal operon can be compared with those of *E. coli* plasmid R773 and *S. aureus* plasmid pI258. For all three, the chromosomal and R773 gene products are considerably more similar to each other than to the pI258 proteins. The similarities are uniform throughout the proteins. The R773 ArsR protein is 75% identical with the chromosomal product, while the pI258 protein is only 31% identical. Comparison of the operator and promoter region of the R773 sequence (44) with the corresponding chromosomal sequence reveals little similarity. While the two ArsR proteins may be repressors, their DNA recognition sequences must be different. The R773 and chromosomal ArsB proteins exhibit 90% identity, and the pI258 protein is 57% identical, to the chromosomal protein. Sofia et al. (39) have suggested that the chromosomal ArsB reading frame begins 21 bp upstream at a GTG codon. There is no obvious ribosome binding site at this site. The alignment of the R773 and chromosomal ArsB proteins suggests that both reading frames begin with the sequence MLLA..., with both utilizing an initiating ATG codon. The putative ribosome binding site for the R773 ArsB protein had been suggested to be AGGAGG (4), and the corresponding sequence in the chromosomal operon is GG-GAGG. The chromosomal and R773 ArsC proteins are 94% identical, while the similarity of either to the pI258 ArsC protein is less than 20%.

**Expression of the cloned *ars* operon confers resistance to arsenite and antimonite.** Sofia et al. (39) had cloned the chromosomal *arsRBC* operon from *E. coli* K-12 strain MG1655 into a  $\lambda$  phage, from which we subcloned the operon into plasmid pUC19, creating plasmid pWPS1. This plasmid was transformed into strain W3110, which is closely related to MG1655 (2), and the levels of resistance to arsenite, antimonite, and arsenate were determined (Fig. 1). Resistance was compared with that of the *arsRDBC* operon of R factor R773 expressed from a similar pBR322-derived plasmid, so that the copy numbers of the two plasmids should be similar. In this construct, the *arsA* gene had been deleted. For unexplained reasons, it has proven difficult to maintain plasmids containing the *arsA* gene in strain W3110. However, its presence would not have allowed a direct comparison of cells producing ArsB proteins, since the ArsA protein increases the level of resistance by forming a complex with the R773 ArsB protein (7a, 9). When expressed from a multicopy plasmid, the cloned chromosomal *arsRBC* operon increased the level of resistance of the host strain to arsenite and antimonite, but no increase in arsenate resistance was observed. In fact, expression of the chromosomal *ars* operon resulted in decreased arsenate resistance (Fig. 1C); this growth phenotype was reproducible but unexplained. The *arsRDBC* genes derived from plasmid R773 gave significantly higher resistance to each metalloid salt, including arsenate. The difference in resistance could be due to different levels of transcription of the two operons, different steady-state levels of the transcripts or proteins, or different efficiencies of the transport systems, questions that will be explored in future studies.

**Effect of deletion of the *ars* operon from the *E. coli* chromosome.** The chromosomal *ars* operon in *E. coli* K-12 strain W3110 was replaced with  $\Delta ars::cam$  as described in Materials and Methods. In a number of repetitions, direct comparisons of the wild-type strain and the deletion strain demonstrated that the deletion strain was 10- to 20-fold more sensitive to arsenite than was the parent (for example, compare Fig. 1A

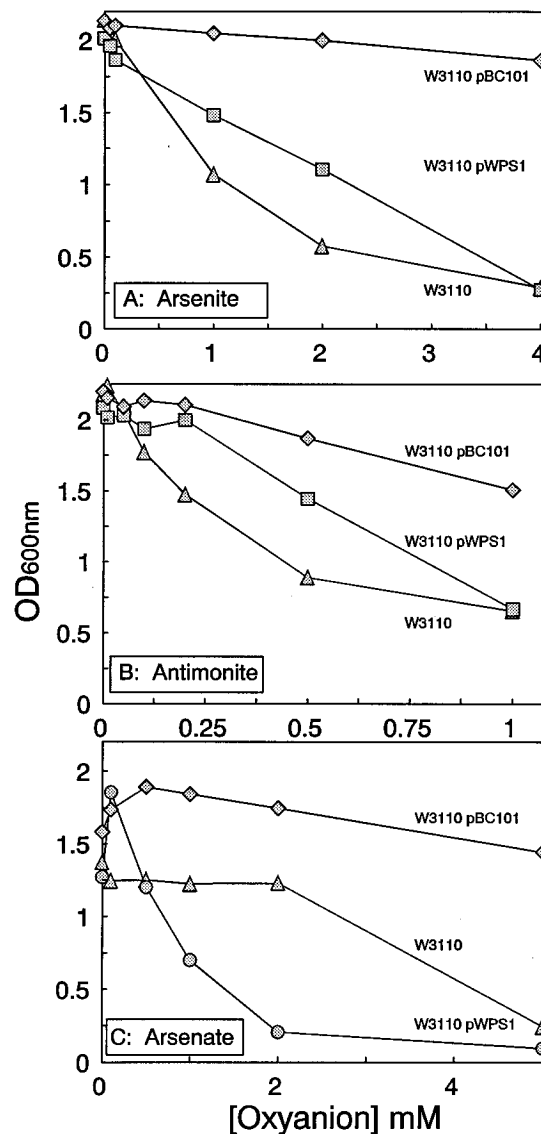


FIG. 1. Resistance to arsenite, antimonite, and arsenate produced by the cloned *arsRBC* operon of *E. coli*. Resistance to arsenite (A), antimonite (B), and arsenate (C) was examined in *E. coli* W3110 with no plasmid ( $\blacktriangle$ ), pBC101 (R773 *arsRDBC*) ( $\blacklozenge$ ), or pWPS1 (chromosomal *arsRBC*) ( $\blacksquare$ ). Resistance was determined in liquid media as described in Materials and Methods. OD<sub>600nm</sub>, optical density at 600 nm.

with Fig. 2A). Wild-type cells were able to grow in the presence of as much as 1 mM sodium arsenite, while growth of the deletion strain was sensitive to 0.05 to 0.1 mM sodium arsenite. The deletion strain was also 5- to 10-fold more sensitive to antimonite (Fig. 2B) and arsenate (Fig. 2C). No increase in sensitivity to tetracycline was detected or HgCl<sub>2</sub> (data not shown). To demonstrate that deletion of the chromosomal *ars* operon increased sensitivity to arsenite in other strains of *E. coli*,  $\Delta ars::cam$  was transferred to strain LE392 $\Delta uncIC$  by transduction with P1 phage. The resulting strain exhibited an increased sensitivity to arsenite very similar to that in AW3110 (data not shown). Thus, the chromosomal *ars* operon is a specific arsenical (antimonial) resistance determinant.

The *ars* deletion strain AW3110 was transformed with plasmid pWPS1 carrying the chromosomal *ars* operon and with

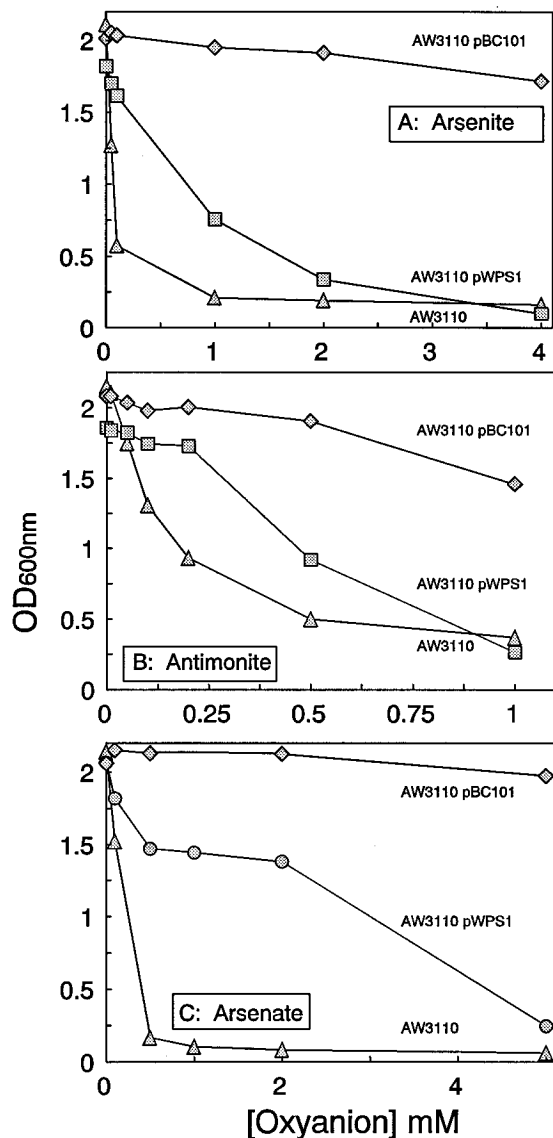


FIG. 2. Effect of chromosomal deletion of the *ars* operon on resistance to arsenite, antimonite, and arsenate. Resistance to arsenite (A), antimonite (B), and arsenate (C) was examined in *E. coli* AW3110 ( $\Delta ars::cam$ ) with no plasmid ( $\blacktriangle$ ), pBC101 (*R773 arsRDBC*) ( $\blacklozenge$ ), or pWPS1 (chromosomal *arsRBC*) ( $\blacksquare$ ). Resistance was determined in liquid media as described in Materials and Methods. OD<sub>600nm</sub>, optical density at 600 nm.

pBC101 carrying the *R773 arsRDBC* operon, and the resistance levels of these strains were compared (Fig. 2). Both plasmids conferred resistance to arsenite (Fig. 2A), antimonite (Fig. 2B), and arsenate (Fig. 2C) in the deletion strain, even though the chromosomal *ars* operon did not increase arsenate resistance in the parental strain. Again the *R773* genes provided higher levels of resistance to all three metalloid salts.

**Energy-dependent arsenite exclusion.** Decreased accumulation of arsenite from cells reflects active extrusion (30). Accumulation of  $^{73}\text{AsO}_2^-$  into cells of *E. coli* LE392 $\Delta uncIC$  and *E. coli* LE392 $\Delta uncIC \Delta ars::cam$  bearing various plasmids was examined (Fig. 3). In the absence of any *ars* genes, cells having the chromosomal *ars* operon accumulated arsenite to a steady-state level of approximately 0.4 nmol/mg of cell protein. In cells with the *ars* operon deleted, the steady-state level of

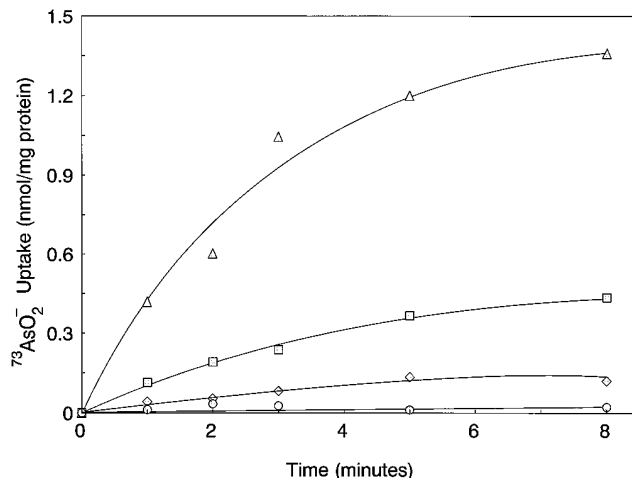


FIG. 3. Energy-dependent arsenite exclusion in cells with deletion of the chromosomal *ars* operon. Accumulation of  $^{73}\text{AsO}_2^-$  was measured in cells as described in Materials and Methods.  $\blacktriangle$ , LE392 $\Delta uncIC \Delta ars::cam$  (pBR322);  $\blacksquare$ , LE392 $\Delta uncIC$  (pBR322);  $\blacklozenge$ , LE392 $\Delta uncIC \Delta ars::cam$  (pBC101);  $\bullet$ , LE392 $\Delta uncIC \Delta ars::cam$  (pWSU1).

arsenite accumulation increased approximately threefold. Expression of either the *R773 arsBC* or *arsABC* genes in cells with the chromosomal operon deleted resulted in a large decrease in accumulation. These results indicate that the products of the chromosomal *ars* operon catalyze arsenite extrusion.

**Inducible expression of *ars* operon in plasmid pWPS1.** The ArsR protein of the *R773 ars* operon has been shown to be a repressor protein (43, 44). In the absence of inducer (arsenite or antimonite), only a basal level of expression of the *R773 ars* operon occurs. When inducer is present, the ArsR dimer is released from the *ars* operator region, resulting in an increase in expression. To determine whether expression of chromosomal *ars* operon is also inducible, cells of *E. coli* W3110 harboring plasmid pWPS1 were grown under inducing conditions. Expression was monitored by immunoblotting with ArsR and ArsC antisera, since, as shown below, the chromosomal ArsR and ArsC proteins were found to cross-react with the antisera to their *R773* homologs. In the absence of inducer, little ArsR protein was observed upon Western blotting (immunoblotting) with the anti-ArsR serum. A basal level of ArsC protein was observed in the absence of inducer, but addition of 10  $\mu\text{M}$  potassium antimonial tartrate as an inducer resulted in increased production of both proteins, demonstrating induction (Fig. 4). It has never been possible to raise antiserum against the ArsB protein, and so it was not possible to determine the level of production of that protein. Since the amount of the ArsB protein is apparently limiting for resistance (28), the observed differences in resistance conferred by the chromosomal and *R773* operons could be due to differential expression of the *arsB* genes. If the inability to increase production of the *R773* ArsB protein by gene dosage is also true of the chromosomal ArsB protein, then this would explain why there is little difference in resistance when that operon is expressed in single copy from the chromosome (Fig. 1) or from a multicopy plasmid (Fig. 2).

Since strain W3110 contains a chromosomal copy of the *ars* operon, production of the Ars proteins in plasmidless strains would be anticipated. However, the levels of those proteins were too low to be detected under the conditions used in the previous experiment. With longer exposure of the chemiluminescent gel, inducible production of the chromosomal ArsC

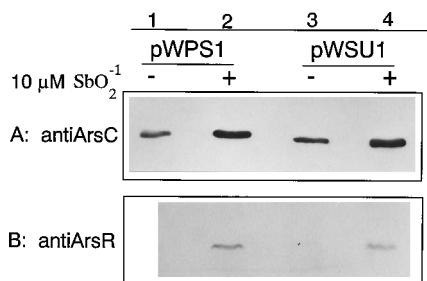


FIG. 4. Expression of the *ars* operon is inducible. Overnight cultures of *E. coli* W3110 bearing plasmid pWSP1 (chromosomal *ars* operon) (lanes 1 and 2) or pWSU1 (R773 *ars* operon) (lanes 3 and 4) were diluted 100-fold into 1 ml of fresh LB medium without inducer (lanes 1 and 3) or with 10  $\mu$ M potassium antimoniate (lanes 2 and 4) and allowed to grow for 6 h with shaking at 37°C. The cells were harvested and boiled in SDS sample buffer, and equal amounts of cell protein were analyzed by SDS-PAGE on 12% acrylamide gels. The proteins were electrophoretically transferred to nitrocellulose membranes and immunoblotted with antisera against the ArsC (A) or ArsR (B) protein from the R773 *ars* operon. The blots were visualized by a chemiluminescent assay with exposure for 15 s (A) and 30 s (B). Purified ArsC and ArsR proteins were used as standards.

protein was observed in cells of strain W3110 (Fig. 5, lanes 1 and 2) but not AW3110 (Fig. 5, lanes 3 and 4). This result confirms that the chromosomal copy of the operon is inducible and that the deletion strain no longer produces the ArsC protein.

**Distribution of the chromosomal *ars* operon.** To determine whether the chromosomal *ars* operon was widespread among laboratory strains of *E. coli*, a variety of strains were analyzed by Southern blotting. Since there are no *HincII* sites in the chromosomal *ars* operon, chromosomal DNA was purified and completely digested with *HincII*. The *EcoRV* fragment containing half of the *arsB* gene and the entire *arsC* gene randomly labeled with [ $\alpha$ - $^{32}$ P]dATP was used as a probe. *E. coli* K-12, CC118, HB101, JM109, TG1, and LE392 $\Delta$ *uncIC* each had hybridizing *HincII* fragments (Fig. 6). Confirming that the  $\Delta$ *ars::cam* recombinant lacked the *ars* operon, no *HincII* fragment from strain LE392 $\Delta$ *uncIC*  $\Delta$ *ars::cam* hybridized with the probe. Other bacteria, including the closely related organism *Salmonella typhimurium*, the gram-negative bacterium *Pseudomonas aeruginosa*, and the gram-positive bacterium *Bacillus subtilis*, did not have hybridizing *HincII* fragments when probed overnight at 65°C.

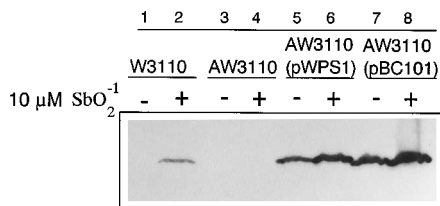


FIG. 5. Inducibility of the chromosomal *ars* operon and effect of chromosomal deletion on production of the ArsC protein. Overnight cultures of *E. coli* W3110 (lanes 1 and 2) or AW3110 ( $\Delta$ *ars::cam*) (lanes 3 to 4) without plasmids (lanes 1 to 4) or bearing plasmid pWSP1 (chromosomal *ars* operon) (lanes 5 and 6) or pWSU1 (R773 *ars* operon) (lanes 7 and 8) were diluted 100-fold into 1 ml of fresh LB medium without inducer (lanes 1, 3, 5, and 7) or with 10  $\mu$ M potassium antimoniate (lanes 2, 4, 6, and 8) and allowed to grow for 4 h with shaking at 37°C. The cells were harvested and boiled in SDS sample buffer, and equal amounts of cell protein were analyzed by SDS-PAGE on a 15% acrylamide gel. The proteins were electrophoretically transferred to nitrocellulose membranes and immunoblotted with antisera against the ArsC protein from the R773 *ars* operon. The blots were visualized by a chemiluminescence assay with exposure for 3 min.

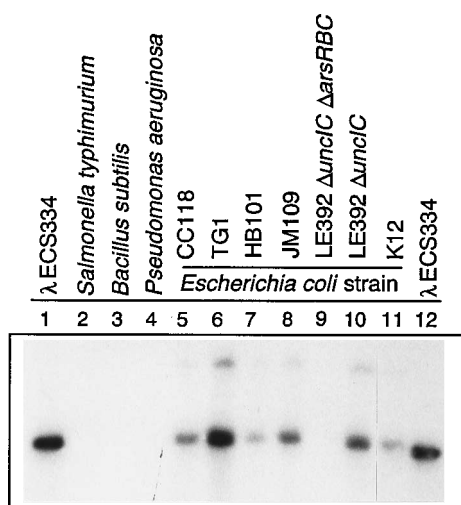


FIG. 6. Distribution of the *ars* operon in bacterial strains. A variety of bacterial strains were analyzed for the presence of the *ars* operon by Southern blotting as described in Materials and Methods. Lanes 1 and 12, *HincII*-digested  $\lambda$ EC27-334 (*arsRBC*); lane 2, *S. typhimurium*; lane 3, *B. subtilis*; lane 4, *P. aeruginosa*; lanes 5 to 11, *E. coli* CC118, TG1, HB101, JM109, LE392 $\Delta$ *uncIC*  $\Delta$ *ars::cam*, LE392 $\Delta$ *uncIC*, and K-12, respectively.

## DISCUSSION

To date, arsenical resistance in *E. coli* has been characterized as a plasmid-mediated process (12, 25, 26, 40). However, plasmidless strains of *E. coli* are intrinsically resistant to arsenite (36), but the basis for this basal level of resistance has been obscure. With the identification of sequences in the *E. coli* chromosome with high similarity to plasmid-encoded *ars* genes, it was suggested that these genes functioned to provide intrinsic resistance to arsenicals and antimonials (39). In this report, we confirm that the chromosomal *ars* operon confers low-level resistance to those oxyanions. First, the cloned genes increase resistance when expressed on a multicopy plasmid. Second, deletion of the *ars* genes from the chromosome results in hypersensitivity to arsenite, and the hypersensitivity is reversed by expression of the operon from a plasmid.

The structure of the chromosomal *ars* operon and its relationship to other *ars* operons are of interest. To date, three plasmid-encoded *ars* operons have been cloned and sequenced. Two, pI258 (15) and pSX267 (32), are from staphylococcal species. Both have three genes, *arsR*, *arsB*, and *arsC*, in order of transcription. Arsenite and antimonite resistance results from the catalytic function of the *arsB* gene product, which transports the oxyanions out of the cells (3, 37). The *arsR* gene product encodes a repressor protein (31), and the *arsC* gene product is an arsenate reductase (16). The third operon was identified on R factor R773 (4, 12), which was derived from an *E. coli* strain isolated from a patient with a urinary tract infection (10). This plasmid-borne operon has five genes, *arsR*, *arsD*, *arsA*, *arsB*, and *arsC* (4, 24, 45). The additional two genes encode an accessory regulatory protein (*arsD*) and an ATPase (*arsA*). The *ArsA* protein binds to the *ArsB* protein (8, 42), forming an arsenite-translocating ATPase complex (7). In the absence of the *arsA* gene, the *ArsB* protein functions similarly to the staphylococcal protein; presumably both act as secondary arsenite porters (7a, 9). The levels of similarity of the chromosomal *ars* gene products with their R773 counterparts (75, 90, and 94%, respectively) make the evolutionary relatedness of the two operons obvious. The lack of *arsD* and *arsA* genes gives the chromosomal *arsRBC* operon a physical

structure more similar to structures of the *ars* operons from plasmids of gram-positive bacteria, even though the gene products exhibit only moderate (57% for ArsB) to poor (19% for ArsC) similarity. It is difficult to reconcile the presence of the *arsD* and *arsA* genes in the R773 operon when they are not present in the other three. This is especially true for the *arsA* gene, since the ArsA protein converts the relatively inefficient proton motive force-coupled ArsB secondary carrier into a more efficient ATP-coupled pump (7a). One possibility is that the acquisition of the additional two genes is a relatively recent event. Another is that the ancestor of all four operons had the *arsD* and/or *arsA* gene but that the genes were lost in three. However, the high degree of relatedness of the chromosomal *ars* operon to the R773 operon compared with the low relatedness of those two to the two staphylococcal operons argues against a single deletion event. If a single deletion had given rise to all three *arsRBC* operons, the chromosomal operon might be expected to have less similarity to the R773 *arsRD-ABC* operon. At present, there is too little information to enable us to draw conclusions; identification of additional *ars* operons might elucidate the evolution of the resistance.

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