AAC Journals.ASM.org

Identification of a Novel Membrane Transporter Mediating Resistance to Organic Arsenic in *Campylobacter jejuni*

Zhangqi Shen,[a] Taradon Luangtongkum,[a,b] Zhiyi Qiang,[c] Byeonghwa Jeon,[a] Liping Wang,[a,d] Qijing Zhang[a]

Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, Iowa, USA[a]; Department of Veterinary Public Health, Chulalongkorn University, Bangkok, Thailand[b]; Department of Food Science and Human Nutrition, Iowa State University, Ames, Iowa, USA[c]; College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, China[d]

Although bacterial mechanisms involved in the resistance to inorganic arsenic are well understood, the molecular basis for organic arsenic resistance has not been described. *Campylobacter jejuni*, a major food-borne pathogen causing gastroenteritis in humans, is highly prevalent in poultry and is reportedly resistant to the arsenic compound roxarsone (4-hydroxy-3-nitrobenzenearsonic acid), which has been used as a feed additive in the poultry industry for growth promotion. In this study, we report the identification of a novel membrane transporter (named ArsP) that contributes to organic arsenic resistance in *Campylobacter*. ArsP is predicted to be a membrane permease containing eight transmembrane helices, distinct from other known arsenic transporters. Analysis of multiple *C. jejuni* isolates from various animal species revealed that the presence of an intact *arsP* gene is associated with elevated resistance to roxarsone. In addition, inactivation of *arsP* in *C. jejuni* resulted in 4- and 8-fold reductions in the MICs of roxarsone and nitarsone, respectively, compared to that for the wild-type strain. Furthermore, cloning of *arsP* into a *C. jejuni* strain lacking a functional *arsP* gene led to 16- and 64-fold increases in the MICs of roxarsone and nitarsone, respectively. Neither mutation nor overexpression of *arsP* affected the MICs of inorganic arsenic, including arsenite and arsenate, in *Campylobacter*. Moreover, acquisition of *arsP* in NCTC 11168 led to accumulation of less roxarsone than the wild-type strain lacking *arsP*. Together, these results indicate that ArsP functions as an efflux transporter specific for extrusion of organic arsenic and contributes to the resistance to these compounds in *C. jejuni*.

Campylobacter, a Gram-negative microaerobic bacterium, is a leading bacterial cause of food-borne diseases, and *Campylobacter* infections account for 400 to 500 million cases of diarrhea each year in developed and developing countries (1). A recent estimate from the CDC indicated that campylobacteriosis accounts for 9% of food-borne illness (over 840,000 cases) every year in the United States (2). *Campylobacter jejuni* and *Campylobacter coli* are the two most important *Campylobacter* species that cause food-borne infections of humans, and raw poultry meat serves as the main source of infection (3). Roxarsone, an organoarsenic compound, has been extensively used as a feed additive in the poultry industry to control bacterial and coccidial infections and improve weight gain, feed utilization, and pigmentation (4). Although it was recently withdrawn from poultry use in the United States, it has been estimated that roxarsone was utilized in approximately 70% of the U.S. broiler production units (4). The concentrations of roxarsone used in feed formulations vary from 22.7 to 45.4 g/ton (5). In animals, roxarsone is excreted into fresh litter and then converted to inorganic arsenate [As(V)] in composted litter via the bioconversion processes (5, 6). The total arsenic concentration in the litter may range from 15 to 48 mg/kg (5–7).

In bacteria, several mechanisms for arsenic detoxification have been characterized, including reduction of As(V) to As(III) by arsenate reductases and extrusion or methylation of As(III) (8–10). The arsenic detoxification systems are encoded by various *ars* genes, including *arsA*, *arsB*, *arsC*, *arsD*, *arsH*, *arsM*, and *arsR*. These *ars* genes can be organized as operons, such as *arsRBC*, *arsRABC*, and *arsRDABC*, or exist singly on bacterial chromosomes (9, 11–17). ArsA functions as an ATPase, which is an energy source for ArsB (18, 19), while ArsB functions as an As(III)-specific transporter (8, 9). As arsenate reductase, ArsC converts As(V) to As(III) in the cytoplasm (8, 20), and then As(III) is extruded by other As(III)-specific transporters (8, 9). ArsD is an arsenic metallochaperone which transfers As(III) to ArsA and increases the rate of arsenic extrusion (10, 21–23). ArsH is an NADPH-flavin mononucleotide oxidoreductase, and the detoxification mechanism is probably through oxidation of arsenite to the less toxic arsenate or reduction of trivalent arsenicals to volatile arsines that escape from cells (24, 25). ArsM is an As(III) *S*-adenosylmethionine methyltransferase, which methylates As(III) to volatile trimethylarsine (10). ArsR functions as a transcription regulator which controls the expression of other *ars* genes (9, 26–29).

Campylobacter is well adapted in the poultry production system and must have means to overcome the toxic effects of arsenic compounds. Recently, a four-gene *ars* operon associated with high-level resistance to inorganic arsenic was identified in *Campylobacter* (9). This operon encodes a putative membrane permease (ArsP), a transcriptional repressor (ArsR), an arsenate reductase (ArsC), and an efflux protein (Acr3). The expression of this operon is directly regulated by ArsR, which binds to the *ars* promoter DNA and controls the expression of the *ars* genes. Insertional mutation of *acr3* and *arsC* revealed that the two genes

Received 2 October 2013 **Returned for modification** 5 November 2013
Accepted 3 January 2014
Published ahead of print 13 January 2014
Address correspondence to Qijing Zhang, zhang123@iastate.edu.
Supplemental material for this article may be found at http://dx.doi.org/10.1128/AAC.02137-13.
Copyright © 2014, American Society for Microbiology. All Rights Reserved.
doi:10.1128/AAC.02137-13

TABLE 1 Key plasmids and bacterial strains used in this study

Bacterial strain or plasmid	Description or relevant genotype	Source or reference
Plasmids		
pUC19	Cloning vector	41
pArsPC	pUC19+*arsP*+*cat*	This study
pRY112	Shuttle vector	42
pRY112+*arsP*	pRY112+*arsP*	This study
pRY112+4*ars*	pRY112 containing the 4-gene *ars* operon	This study
Strains		
DH5α	Plasmid propagation *E. coli* strain	Invitrogen
NCTC 11168	Wild-type *C. jejuni*	43
11168+*arsP*	NCTC 11168 derivative, pRY112+*arsP*	This study
11168+4*ars*	NCTC 11168 derivative, pRY112 + 4*ars*	This study
CB5-28	Wild-type *C. jejuni*	9
CB5-28 Δ*arsC*	CB5-28 derivative, Δ*arsC*::Kan^r	9
CB5-28 Δ*arsP*	CB5-28 derivative, Δ*arsP*::*cat*	This study

TABLE 2 Key primers used in this study

Primer	Sequence (5′→3′)
S1730-F	TATTTACTGAGCTTTCTGTACTTT
S1730-R	TAGCCACGCCTAAAATAATC
Ars1-F	CATTCATTTTAGAGTTATTGCGTATAAAACATACT
ArsPN-R	CCATGTTTAAGAGCTCTTGTAGATCAC
ArsPT-F	CATCCATATACAAACTTCCAATACCCC
ArsPT-R	CCTGCGGAAAATACTTCGATATCTATGCC
ArsPW-F	ACATACTGGTTCTAAATTTCTGATCAAACG
ArsPW-R	AATCCAGAAAGCAAGGACTATCAACCT

contribute to arsenite and arsenate resistance but had no effects on the MIC of roxarsone in *Campylobacter* (9). However, the function of ArsP has not been characterized. In general, the molecular mechanisms directly involved in organic arsenic (roxarsone) resistance have not been described for any bacteria. In this study, we identify ArsP as a specific mechanism for organic arsenic resistance in *Campylobacter*.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The key bacterial strains and plasmids used in this study are listed in Table 1. *Escherichia coli* DH5α used for genetic manipulation was grown in Luria-Bertani (LB) broth or on LB agar supplemented with kanamycin (30 μg/ml), chloramphenicol (10 μg/ml), or ampicillin (100 μg/ml). *C. jejuni* strains were cultured on Mueller-Hinton (MH) agar or in MH broth at 42°C microaerobically (5% O_2, 10% CO_2, and 85% N_2). When needed, kanamycin (30 μg/ml) or chloramphenicol (4 μg/ml) was used to supplement the media.

Chemical compounds and antibiotics. The chemicals and antibiotics used in this study were purchased from Sigma-Aldrich Co. LLC (arsenite, arsenate, nitarsone, arsanilic acid, chloramphenicol, kanamycin, streptomycin, and ampicillin) and Fisher Scientific Inc. (roxarsone and carbarsone).

Bioinformatics. Protein Basic Local Alignment Search Tool (BLASTP) was used to search ArsP homologues in databases of nonredundant protein sequences. Constraint-based Multiple Protein Alignment Tool (COBALT) (30) was used to carry out multiple-sequence alignment and construct the phylogenetic tree. A biological sequence alignment editor (BioEdit) was used to output the multiple-sequence alignment result. TopPred 0.01 (31), MEMSAT-SVM (32), and TMHMM 2.0 (33) were used to predict the transmembrane helices (TMH) of ArsP. The visual representation of ArsP transmembrane topology was demonstrated by TMRPres2D (34). The sequence logo was generated using WebLogo, version 2.8.2 (35).

Antimicrobial susceptibility tests. The MICs of various arsenic compounds against *C. jejuni* strains were determined using the agar dilution or broth dilution antimicrobial susceptibility testing methods according to the protocol from the CLSI (36). MICs of azithromycin, ciprofloxacin, erythromycin, gentamicin, tetracycline, florfenicol, nalidixic acid, telithromycin, and clindamycin were determined using commercially prepared Sensititre broth microdilution plates (Trek Diagnostic Systems, Cleveland, OH). Every MIC test was repeated at least three times. *C. jejuni* ATCC 33560 was used as a quality control organism in this study.

Construction of the *arsP* mutant. Primers AspT-F and ArsPT-R (Table 2) were used to amplify a 1.8-kb *arsP* fragment with two PsiI sites in the middle region of the fragment. The PCR fragment was cloned into the pUC19 in the SmaI sites, resulting in the construction of pArsP. The chloramphenicol resistance *cat* cassette amplified from pUOA18 using the Phusion HighFidelity DNA polymerase (NEB) (37) was ligated into the PsiI site of *arsP* in pArsP to yield the pArsPC plasmid. Suicide vector pArsPC was introduced into *C. jejuni* CB5-28 using electroporation. Transformants were selected on MH agar containing chloramphenicol at 4 μg/ml. The insertion of *cat* cassette into the *arsP* gene was confirmed by PCR analysis.

Cloning of the *arsP* and the *ars* operon into *C. jejuni* NCTC 11168. The entire *arsP* gene, including its promoter region, was amplified from strain CB5-28 by PCR using primers Ars1-F and ArsPN-R (Table 2). The PCR product was cloned into the SmaI-digested pRY112 plasmid to construct pRY112+*arsP*. pRY112+*arsP* was sequenced, and it was confirmed that no mutations in the cloned sequence had occurred. The shuttle plasmid, pRY112+*arsP*, was transferred into NCTC 11168 by conjugation. The resulting strain was named 11168+*arsP*. The entire *ars* operon, including *arsP*, *arsR*, *arsC*, and *acr3*, was amplified from strain CB5-28 by PCR using primers ArsPW-F and ArsPW-R. The PCR product was cloned into the SmaI-digested pRY112 plasmid to construct pRY112+4*ars*. pRY112+4*ars* was sequenced, and it was confirmed that no mutations in the cloned sequence had occurred. The shuttle plasmid pRY112+4*ars* was transferred into NCTC 11168 by conjugation. The resulting strain was named 11168+4*ars*.

PCR and sequence analysis of *arsP*. To detect the distribution of *arsP* with in various *C. jejuni* isolates, the S1730-F and S1730-R primers (Table 2) were used to amplify an intragenic region of the *arsP* gene. Since the *arsP* gene in some strains (e.g., *C. jejuni* NCTC 11168) is a pseudogene, if a positive PCR product was obtained from the strain with low MIC for roxarsone, the whole coding region of *arsP* was amplified using another pair of primers, ArsPW-F and ArsPW-R, and the product was then sequenced in the DNA facility at Iowa State University. PCR was performed in a volume of 50 μl containing 0.2 μM primers, 250 μM deoxynucleoside triphosphates (dNTPs), and 1.25 U of TaKaRa *Ex Taq* polymerase. An annealing temperature of 50°C and an elongation time of 1 min were used for the primer pair S1730-F and S1730-R, while an annealing temperature of 55°C and an elongation time of 4 min were used for the primer pair ArsPW-F and ArsPW-R.

Roxarsone accumulation assay. To determine if ArsP contributes to reduce intracellular concentration of roxarsone, an accumulation assay was conducted using *C. jejuni* NCTC 11168 and 11168+*arsP*. NCTC 11168 and 11168+*arsP* were inoculated on MH agar plates and incubated microaerobically for 24 h. Bacterial cells were collected from the plates, inoculated into fresh MH broth (200 ml), and then incubated at 42°C with shaking at 160 rpm. After 4 to 5 h of incubation, the cells were harvested by centrifugation, washed once in phosphate-buffered saline (PBS), and then resuspended in PBS to $10^{10\sim11}$ CFU/ml. Three different tubes were prepared for each sample. After incubation at 37°C for 10 min, roxarsone was added to a final concentration of 500 μg/ml. Aliquots (1.0 ml) were taken after 15 min and immediately mixed with 5.0 ml of ice-cold PBS. Bacterial cells were harvested by centrifugation at 4°C and 6,000 × *g* for 5 min and washed twice with 10 ml of ice-cold PBS. The cells were resuspended in 0.5

TABLE 3 Roxarsone MIC distributions in *C. jejuni* isolates of different origins

Isolate source	Total no.	No. of isolates inhibited by ROX concn (μg/ml)								MIC_{50}/MIC_{90} (μg/ml)
		4	8	16	32	64	128	256	512	
Chicken	35	2	11	7	4	11	0	0	0	16/64
Human	27	5	4	4	5	5	3	1	0	32/128
Sheep	35	1	23	6	3	2	0	0	0	8/32
Turkey	34	0	7	18	7	0	1	1	0	16/32

ml of PBS and then disrupted by ultrasonication. The supernatant was taken after centrifugation at 15,000 × *g* for 5 min and filtered with a 0.45-μm filter. High-performance liquid chromatography (HPLC) analysis was performed on a Beckman Coulter 126 HPLC, equipped with a photodiode array detector (model 168) and a model 508 autosampler (Beckman Coulter, Inc., Brea, CA). The mobile phase was monopotassium phosphate (0.05 M)-acetic acid (10%)-methanol (90:7:3, vol/vol/vol) at a flow rate of 1.0 ml/min. A universal reversed-phase Supelcosil ABZ$^+$Plus column (150 mm by 4.6 mm by 5 μm; Supelco, Bellefonte, PA) was used at room temperature. A standard curve was prepared by measuring the peak area from PBS containing serially diluted roxarsone and used to determine the concentration of roxarsone in the samples.

RESULTS

Arsenic resistance varies in *Campylobacter* strains of different origins. To determine if arsenic resistance in *Campylobacter* is associated with the origin of isolation, we examined the MICs of various arsenic compounds in *C. jejuni* isolates from different animal species. In total, 131 *C. jejuni* isolates (35 from chickens, 27 from humans, 35 from sheep, and 34 from turkeys) were evaluated by the agar dilution method (Tables 3, 4, and 5). In general, MICs of roxarsone, arsenite, and arsenate ranged from 4 to 256 μg/ml, 1 to 256 μg/ml, and 16 to >1,024 μg/ml, respectively, and the MIC_{50} and MIC_{90} of the sheep isolates were lower than those of the chicken, human, and turkey isolates (Tables 3, 4, and 5). Statistical analysis with the Kruskal-Wallis test (nonparametric analysis of variance [ANOVA]) indicated that the MICs of *C. jejuni* from different animal species are significantly different in terms of resistance to roxarsone ($P < 0.005$), arsenite ($P < 0.002$), and arsenate ($P < 0.0001$). Furthermore, Dunn's multiple-comparison test indicated that the MICs of roxarsone for the sheep isolates are significantly lower than those for the human ($P < 0.01$), chicken ($P < 0.05$), and turkey ($P < 0.05$) isolates, the MICs of arsenite for the sheep isolates were significantly lower than those for the chicken isolates ($P < 0.001$), the MICs of arsenate for the sheep isolates were significantly lower than those for the chicken ($P < 0.001$) and turkey ($P < 0.001$) isolates, and the MICs of roxarsone, arsenite, and arsenate for the human, chicken, and turkey isolates were not significantly different ($P > 0.05$).

Genetic features of *arsP*. *arsP* (*cje1730*) is the first gene in the 4-gene *ars* operon and encodes a putative transmembrane permease (315 amino acids [aa]) (Fig. 1A). The transmembrane helices (TMH) of ArsP were predicted by using different algorithms, including TopPred 0.01, MEMSAT-SVM, and TMHMM 2.0. Generally, the three methods predicted similar topologies for ArsP. Results from MEMSAT-SVM and TMHMM 2.0 indicated that ArsP has eight putative TMH, while TopPred 0.01 failed to predict the third TMH (Fig. 2). All three methods predicted a large hydrophilic loop (~60 aa) in the central portion (between the fourth and fifth helices) of the protein (Fig. 2). The large central hydrophilic loop is predicted to be outside the inner membrane, facing the periplasmic space. Thus, the predicted topology of ArsP is quite different from those of ArsB and Acr3. Additionally, ArsP shares little sequence homology with ArsB or Acr3, which are transporters for As(III). According to the transporter classification database (TCDB; http://www.tcdb.org), ArsP (TCID: 9.B.28.1.1) belongs to Duf318 family of putative permeases. The database indicates that none of the members of the Duf318 family has been functionally characterized. These observations suggest that ArsP may have a distinct substrate specificity. Based on BLAST search of the GenBank database, ArsP homologues were found in 27 bacterial and archaeal genera (see Fig. S1 in the supplemental material for phylogenetic tree), including at least 68 species. The amino acid lengths of the ArsP homologues range from 294 to 365 aa, and TMHMM 2.0 predicted that all of them contain similar TMH with large hydrophilic loops between the fourth and fifth TMH, ranging from 37 to 109 amino acids in length. Multiple-sequence alignment indicated that the ArsP homologues are highly conserved in the transmembrane regions but quite diverse in the central loop region (see Fig. S2 in the supplemental material). Using WebLogo, a highly conserved signature motif, TPFCSCSTIP, located in the second transmembrane domain was identified among the ArsP homologues (Fig. 3). Since *arsP* is in the *ars* operon, we hypothesized that ArsP plays a role in arsenic resistance in *Campylobacter*.

Correlation of *arsP* with increased resistance to roxarsone in different *C. jejuni* strains. To examine the association between *arsP* and the enhanced resistance to roxarsone, the presence of *arsP* in different *C. jejuni* isolates was determined using PCR. In total, 54 *C. jejuni* isolates (19 from humans, 21 from chickens, and

TABLE 4 Arsenite MIC distributions in *C. jejuni* isolates of different origins

Isolate source	Total no.	No. of isolates inhibited by As(III) concn (μg/ml)									MIC_{50}/MIC_{90} (μg/ml)
		1	2	4	8	16	32	64	128	256	
Chicken	35	0	2	1	8	9	7	8	0	0	16/64
Human	27	2	0	3	9	6	0	5	2	0	8/64
Sheep	35	0	0	8	23	0	4	0	0	0	8/32
Turkey	34	0	1	10	9	5	2	6	0	1	8/64

TABLE 5 Arsenate MIC distributions in *C. jejuni* isolates of different origins

Isolates	Total no.	No. of isolates inhibited by As(V) concn (μg/ml)								MIC_{50}/MIC_{90} (μg/ml)
		16	32	64	128	256	512	1,024	>1,024	
Chicken	35	0	5	10	1	9	6	4	0	256/1,024
Human	27	6	6	4	1	4	4	0	2	64/512
Sheep	35	6	19	2	2	6	0	0	0	32/256
Turkey	34	1	4	4	13	3	3	3	3	128/1,024

14 from turkeys) were used. Statistical analysis of the PCR and MIC data with Wilcoxon rank sum test with continuity correction indicated that the presence of an intact *arsP* gene is significantly ($P < 0.001$) associated with elevated resistance to roxarsone (Table 6). The PCR results also showed that an *arsP* gene is not always linked with the *acr3* gene (member of 4-gene operon) (Table 6). Six of the strains (CT4-17, F1587, 11168, W28752, W56246, and X39768) are negative for both *arsP* and *acr3* as determined by PCR amplification, while only 3 of the strains (CB5-28, RM1221, and S13530) are positive for both *arsP* and *acr3*. Notably, most of the strains contain either *arsP* or *acr3* (Table 6). The association of *arsP* with the elevated MIC of roxarsone suggested its possible contribution to roxarsone resistance in *C. jejuni*.

Role of *arsP* in the arsenic resistance. To determine the role of *arsP* in arsenic resistance in *C. jejuni*, insertional mutagenesis was used to construct an isogenic mutant. The *arsP* mutant was compared with the parent strain CB5-28 and CB5-28 Δ*arsC* for susceptibility to arsenic compounds using the agar dilution method. According to MIC results from the agar dilution method, inactivation of *arsP* resulted in a 4-fold reduction in the MICs of roxarsone, arsenite, and arsenate, respectively, while inactivation of the downstream *arsC* gene resulted in 4-fold and 32-fold reductions in the MICs of arsenite and arsenate, respectively, but did not affect the MIC of roxarsone (Table 7). This finding suggests that *arsP* is associated with roxarsone resistance in *Campylobacter*. Since the *arsP* mutation might cause a polar effect on the expression of the downstream genes in the *ars* operon, we further analyzed the function of *arsP* by cloning this gene into NCTC 11168, which lacks *arsP* and does not contain a functional *ars* operon (Fig. 1). According to the MIC results, acquisition of the single *arsP* gene in NCTC 11168 resulted in an 16-fold increase in the MICs of roxarsone but had no effects on the MICs of arsenite or arsenate (Table 7), indicating that *arsP* specifically contributes to the resistance to roxarsone but not to arsenite or arsenate. When the entire *ars* operon was cloned into NCTC 11168 (Fig. 1), it resulted in 8-fold, 8-fold, and 2-fold increases in the MICs of roxarsone, arsenite, and arsenate, respectively. In addition to roxarsone, we also examined the MICs of three other organoarsenic compounds: arsanilic acid, carbarsone, and nitarsone. The results indicated that inactivation of *arsP* in CB5-28 resulted in 2-fold and 8-fold reductions in the MICs of arsanilic acid and nitarsone, respectively, but did not affect the MIC of carbarsone (Table 7). In addition, acquisition of the single *arsP* gene in NCTC 11168 resulted in 2-fold and 64-fold increases in the MICs of arsanilic acid and nitarsone, respectively, but did not affect the MIC of carbarsone (Table 7). These results demonstrate the specific and varied role of ArsP in the resistance to organoarsenic compounds.

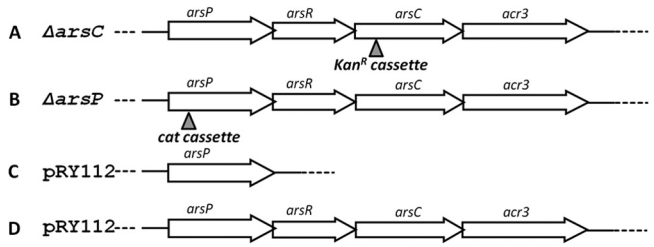

FIG 1 Diagrams showing the genomic organization of *arsP* and its flanking genes as well as cloning of the *ars* genes into *C. jejuni* NCTC 11168. (A) The *ars* operon in *C. jejuni* CB5-28 and inactivation of *arsC* by insertion of a kanamycin resistance cassette. (B) The *ars* operon in *C. jejuni* CB5-28 and inactivation of *arsP* by insertion of a choramphenicol resistance cassette. (C) Cloning of the single *arsP* gene from CB5-28 to shuttle plasmid pRY112, which was then transferred into NCTC 11168 by conjugation. (D) Cloning of the entire *ars* operon from CB5-28 to shuttle plasmid pRY112, which was then transferred into NCTC 11168 by conjugation.

ArsP did not confer resistance to the other antibiotics. To examine if ArsP contributes to the resistance of other antimicrobials, we compared the susceptibilities of the wild-type and genetically modified *Campylobacter* strains to azithromycin, ciprofloxacin, erythromycin, gentamicin, tetracycline, florfenicol, nalidixic acid, telithromycin, and clindamycin using commercially prepared Sensititre broth microdilution plates. The results showed no differences between the wild type and the genetically modified strains in the susceptibilities to these compounds (data not shown), indicating that *arsP* does not confer resistance to these tested antibiotics.

The *arsP* gene is not inducible by roxarsone. To determine if the transcriptional level of *arsP* gene is inducible by roxarsone, *C. jejuni* CB5-28 was cultured in MH broth with different concentrations of roxarsone. The transcriptional level of *arsP* in cultures supplemented with or without roxarsone did not differ as measured by quantitative reverse transcription-PCR (qRT-PCR) (data not shown). This result suggests that *arsP* is not inducible by roxarsone.

ArsP reduces intracellular accumulation of roxarsone. An accumulation assay using HPLC was performed to assess whether ArsP functions as an efflux transporter for roxarsone. The wavelength was 337 nm and the retention time was 5.2 min for roxarsone (Fig. 4B). According to the standard curve, a linear regression equation was obtained: $y = (x - 287.54)/11{,}176$, with an R^2 of 0.9999, where y is the concentration of roxarsone and x is the peak area of the sample. Using this equation, we were able to measure the concentrations of roxarsone in different samples. The NCTC 11168 wild type, which does not have a functional *arsP* gene, accumulated 44.8% more roxarsone than 11168+*arsP* (containing an intact *arsP* gene) (Fig. 4A). This result suggests that ArsP functions as an efflux transporter, extruding roxarsone out of *Campylobacter* cells.

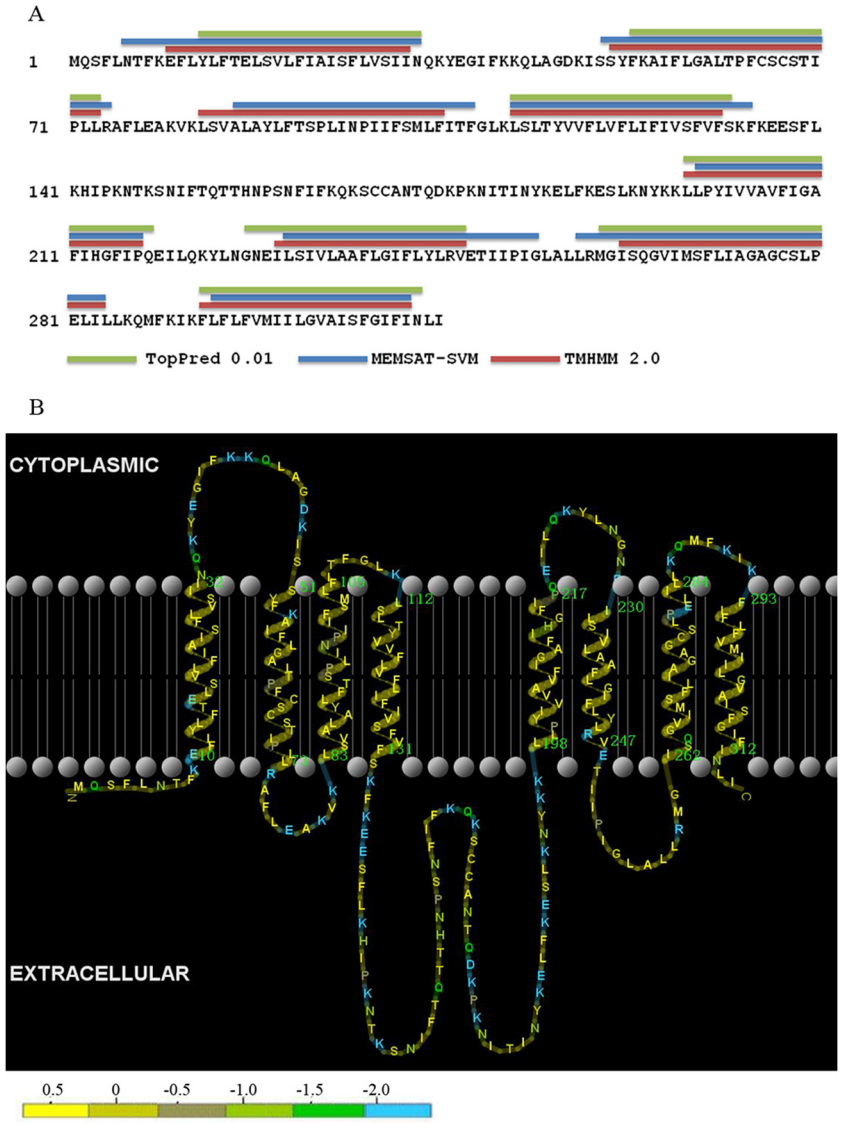

FIG 2 Predicted membrane topologies of ArsP. (A) The prediction of TMH was based on the amino acid sequence of ArsP in *C. jejuni* CB5-28 and was done by using three algorithms, including TopPred 0.01(green), MEMSAT-SVM (blue), and TMHMM 2.0 (red). (B) Visual representation of ArsP transmembrane topology. This is based on the TMHMM prediction and is shown by TMRPres2D. The hydrophobic potential of the 20 amino acid residue types is illustrated by different colors. The model predicts that a large hydrophilic loop (~60 aa) is in the central portion (between the fourth and fifth helices) of ArsP.

DISCUSSION

The results from this study identify ArsP as a novel membrane transporter mediating resistance to organic arsenic in *C. jejuni*. This conclusion is supported by multiple pieces of evidence. The presence of ArsP is associated with elevated MICs of roxarsone (Table 6). Inactivation of *arsP* resulted in 2-, 8-, and 4-fold reductions in the MICs of arsanilic acid, nitarsone, and roxarsone, respectively, while cloning of *arsP* into a *C. jejuni* strain lacking a functional *arsP* showed 2-fold, 64-fold, and 16-fold increases in the MICs of arsanilic acid, nitarsone, and roxarsone, respectively, but had no effect on the MICs of inorganic arsenic compounds and antibiotics. Additionally, ArsP reduced intracellular accumulation of roxarsone in *C. jejuni*, and the presence of the *arsP* gene is associated with elevated resistance to roxarsone in various strains. These results indicate that ArsP functions as a resistance mechanism specific for organic arsenic. This finding enriches our knowledge of various mechanisms for arsenic resistance in *Campylobacter*. Based on these results and previously known information regarding arsenic resistance in *Campylobacter*, a model showing the various arsenical detoxification mechanisms is presented in Fig. 5.

Due to the use of organoarsenics in poultry production, *Campylobacter* spp. in poultry must be able to deal with the toxicity and selective pressure from arsenic compounds. A previous study indicated that the *Campylobacter* isolates from conventional poultry products had significantly higher roxarsone MICs than those isolated from antimicrobial-free poultry products, suggesting the association of the roxarsone use in conventional poultry facilities with the development of roxarsone resistance in *Campylobacter* spp. in poultry (38). In this study, we demonstrated that the pres-

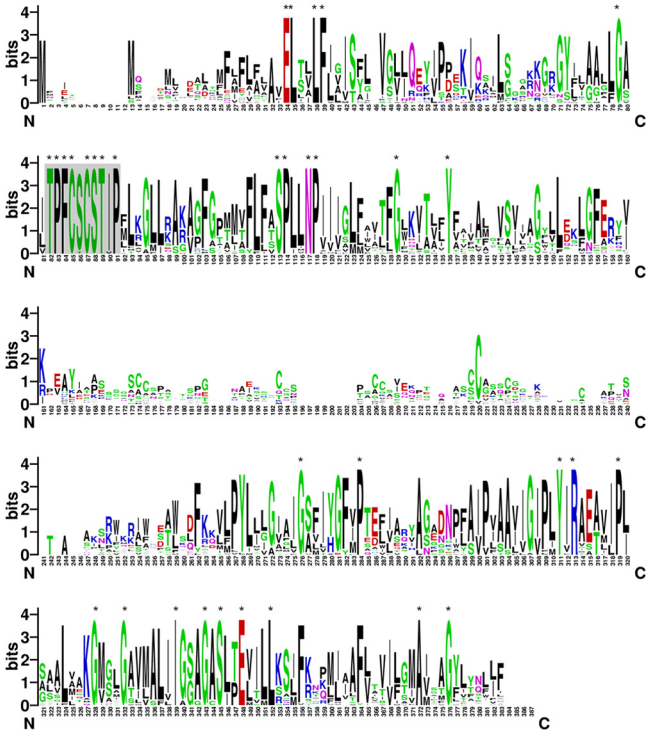

FIG 3 Identification of a signature motif among ArsP homologues by WebLogo. The sequence logo was constructed from the alignment of 27 ArsP homologues. The letter size is proportional to the degree of amino acid conservation. The position of signature motif TPFCSCSTIP is shaded gray.

ence of an intact *arsP* gene is associated with increased resistance to roxarsone in various *C. jejuni* isolates (Table 6). This observation plus the findings from the mutagenesis and cloning experiments established ArsP as a key resistance mechanism for roxarsone, providing a molecular explanation for the prevalence of roxarsone-resistant *Campylobacter* in poultry. Although *arsP* is found to be in the same operon as *arsC* and *acr3* in certain *C. jejuni* strains, it is functionally distinct from the other two genes, which confer resistance to arsenite and arsenate (9). Additionally, it was found that *arsP* does not have to be associated with *arsC* and *acr3*, which was also shown in a recent study by Noormohamed and Fakhr on the prevalence of *ars* genes in *C. jejuni* isolates from retail meats (39). These findings suggest that *arsP* evolved differently from other *ars* genes. Interestingly, the study by Noormohamedemail and Fakhr (39) reported a much higher detection rate (94.7%) of *arsP* in their *C. jejuni* isolates than that reported in this study (~50%). The difference in the *arsP* detection rates is probably due to the different origins of the isolates.

In this study, we tested the MICs of arsenic compounds for the *C. jejuni* isolates from chickens, turkeys, humans, and sheep. An interesting finding is that the MICs of roxarsone for the sheep isolates were significantly lower than those for isolates from humans, chickens, and turkeys. This is probably due to the fact that the sheep industry does not use roxarsone as a feed additive, while roxarsone was extensively used on chicken and turkey farms in the United States. Although the use of roxarsone was banned in the European Union in 1999 and the manufacturer voluntarily suspended sale of roxarsone in the United States in 2011, roxarsone is still approved for use in poultry and livestock industry in many other countries (http://www.fda.gov/AnimalVeterinary/SafetyHealth/ProductSafetyInformation/ucm257540.htm). Interestingly, human *C. jejuni* isolates showed a level of roxarsone resistance similar to that of the turkey and chicken isolates. This could be explained by the fact that poultry is the main reservoir for human *C. jejuni* infections.

Notably, ArsP shows substrate specificity, as mutation of the gene or acquisition of the gene only affected the MICs of or-

TABLE 6 Presence of *arsP* detected by PCR and its association with elevated resistance to roxarsone

Strain	MIC (μg/ml)	Presence of intact *arsP*[a]	Source
CB3-15	8	−	Chicken
CB3-18	8	−	Chicken
CB3-23	8	−	Chicken
CB3-6	8	−	Chicken
CB3-9	8	−	Chicken
CB7-22	8	−	Chicken
CT3-19	16	−	Turkey
CT3-7	8	−	Turkey
CT4-10	16	−	Turkey
CT4-15	16	−	Turkey
CT4-17	32	−	Turkey
CT4-4	16	−	Turkey
CT4-6	16	−	Turkey
CT5-1	16	−	Turkey
CT5-10	16	−	Turkey
CT5-12	16	−	Turkey
CT5-2	8	−	Turkey
CT5-9	8	−	Turkey
E46972	128	−	Human
F1587	4	−	Human
M33323	8	−[b]	Human
M36292	32	−	Human
M402	16	−	Human
M76297	16	−	Human
NCTC 11168	8	−[b]	Human
T37957A	32	−	Human
W28752	8	−	Human
W56246	4	−	Human
X39768	128	−	Human
CB1-10	64	+	Chicken
CB1-15	64	+	Chicken
CB1-17	32	+	Chicken
CB1-19	64	+	Chicken
CB1-9	64	+	Chicken
CB2-12	16	+	Chicken
CB2-13	16	+	Chicken
CB2-5	32	+	Chicken
CB2-8	16	+	Chicken
CB2-9	32	+	Chicken
CB3-3	64	+	Chicken
CB4-1	64	+	Chicken
CB4-4	128	+	Chicken
CB5-28	32	+	Chicken
RM1221	64	+	Chicken
CT3-2	32	+	Turkey
CT4-20	128	+	Turkey
H307369	32	+	Human
H49024	64	+	Human
M37523	32	+	Human
S13530	64	+	Human
W64861	128	+	Human
X77136	16	+	Human
cjs47645	64	+	Human
Clev9100	64	+	Human

[a] −, absence of genes by PCR detection; +, presence of genes by PCR detection.
[b] Presence of frameshift mutations in *arsP*.

TABLE 7 MICs of roxarsone, arsenite, arsenate, arsanilic acid, carbarsone, and nitarsone in various *C. jejuni* constructs

Strain	MIC (μg/ml)[a]					
	Roxarsone	Arsenite	Arsenate	Arsanilic acid	Carbarsone	Nitarsone
NCTC 11168	8	16	1,024	1,250	5,000	4
11168+*arsP*	128 (↑16)	16	1,024	2,500 (↑2)	5,000	256 (↑64)
11168+*4ars*	64 (↑8)	128 (↑8)	2,048 (↑2)	2,500 (↑2)	5,000	128 (↑32)
CB5-28	32	64	2,048	2,500	5,000	32
CB5-28 Δ*arsP*	8 (↓4)	16 (↓4)	512 (↓4)	1,250 (↓2)	5,000	4 (↓8)
CB5-28 Δ*arsC*	32	16 (↓4)	64 (↓32)	2,500	5,000	32

[a] The numbers in parentheses indicate fold changes over the wild-type control, either increased (↑) or decreased (↓).

ganic arsenic and did not have any effect on the MICs of inorganic arsenic and other antibiotics (Table 7). Even among the organoarsenic compounds examined in this study, ArsP was more effective against roxarsone and nitarsone than arsanilic acid and had no effect on carbarsone (Table 7). The difference in the effects is likely due to the structural differences among the organoarsenic compounds. Specifically, the structural differences among the tested compounds lie in the modifications of the benzene ring by a nitro group (roxarsone and nitarsone), an amino group (arsanilic acid), or a carbamide group (carbarsone). Thus, it appears that ArsP functions more efficiently with phenylarsenic compounds modified by nitro groups than with those modified by other groups. It should be pointed out that we tested only a limited number of organoarsenic compounds and did not evaluate the ability of ArsP to transport 4-hydroxy-3-nitrobenzene or related nitroaromatic compounds. Considering the wide distribution of ArsP homologues in various bacterial organisms, it is possible that ArsP has a function beyond organoarsenic resistance. This possibility remains to be determined in future studies.

Compared to other known arsenic efflux transporters (such as ArsB and Acr3), ArsP is structurally unique in having 8 transmembrane domains and a large hydrophilic and centrally located loop (Fig. 2). These structural features may have determined the substrate specificity of ArsP. Interestingly, the ArsP in *C. jejuni* contains 5 cysteines, which are in positions 65, 67, 169, 170, and 277 (Fig. 2A). 169C and 170C are located in the nonconserved central loop region, while 65C and 67C are located in the highly conserved signature motif of the second transmembrane domain (Fig. 2; see also Fig. S2 in the supplemental material). It is unknown if they are redox reactive and if they are involved in the interaction with the substrates. Further structural and chemical analyses are required to answer these questions.

Although ArsP contributes to organoarsenic resistance, its expression is not inducible by roxarsone. Previously it was revealed that *arsP* is regulated by ArsR, which is encoded in the *ars* operon that includes *arsP*, *arsC*, and *acr3* (9). The induction of the *ars* operon is through the inhibition of ArsR binding to the promoter DNA, which is mediated by conformational change in ArsR triggered by arsenite binding (40). Thus, arsenite is the true inducer of ArsR, and the arsenate-mediated induction is via bioconversion of arsenate to arsenite by ArsC reductase in *Campylobacter*. In this study, the presence of roxarsone in culture did not induce the expression of *arsP*. This result indicates that roxarsone does not interact with ArsR and does not directly induce the *ars* operon. However, roxarsone can be converted to inorganic arsenic in chicken litter through biological processes (5) and is a potential indirect inducer for the *ars* operon in the poultry production environment. Additionally, the MICs of roxarsone for the isolates containing the *arsP* genes range from 16 to 128 μg/ml (Table 6). The wide range of MICs suggests the differential expression of *arsP* or the existence of unknown regulatory mechanisms for *arsP* in those isolates. This possibility remains to be determined in future studies.

The results from the roxarsone accumulation assay (Fig. 4) suggest that AsrP functions as an efflux transporter. This finding is consistent with the predicted function of ArsP as a permease. However, the energy source required for ArsP to transport organic arsenic is unknown at this stage. According to the TCDB database (http://www.tcdb.org), ArsP is distantly related to both primary (ABC transporter ThiW; 3.A.1.26.2) and secondary (TrpP;

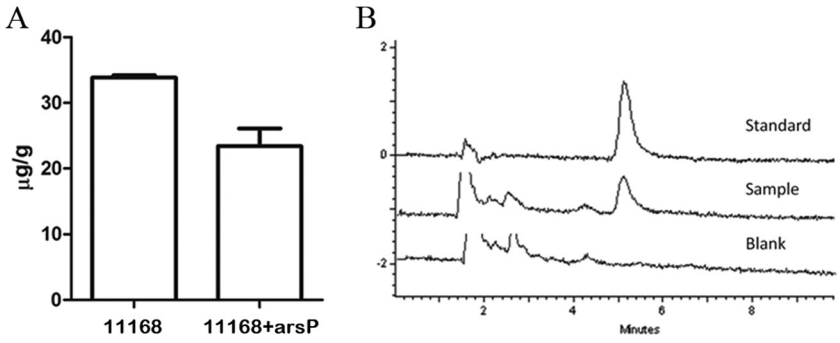

FIG 4 Intracellular accumulation of roxarsone as measured by HPLC. (A) Accumulation of roxarsone in wild-type NCTC 11168 and 11168+*arsP*. Each bar represents the mean and standard deviation of triplicate samples in one representative experiment ($P < 0.02$). (B) Representative chromatogram of the HPLC assay to determine roxarsone accumulation in *Campylobacter*. The peak at 5.2 min represents roxarsone. The standard is PBS buffer with added roxarsone. The sample is a PBS cell lysate of NCTC 11168 treated with roxarsone. The blank control is a PBS cell lysate of NCTC 11168.

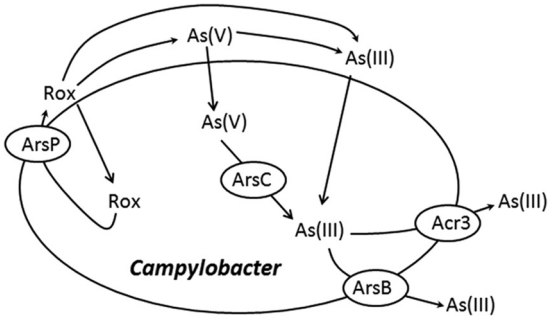

FIG 5 Model illustrating the currently known arsenical detoxification mechanisms in *Campylobacter*. *Campylobacter* has an arsenic reductase (ArsC), which converts arsenate to arsenite, two types of arsenite transporters (ArsB and Acr3), which extrude arsenite, and the ArsP transporter, which specifically extrudes roxarsone (Rox) out of the cells.

2.A.88.4.1) transporters. A motif search did not identify an ATP-binding site in ArsP, but this does not exclude the possibility that ArsP utilizes ATP by partnering with a ATPase. Additionally, we compared the MICs of roxarsone and nitarsone in *C. jejuni* CB5-28 in the presence and absence of carbonyl cyanide m-chlorophenylhydrazine (CCCP), an inhibitor of the membrane proton gradient, and found that the presence of CCCP made no difference in the MICs (data not shown). This result suggests that ArsP may not utilize the proton gradient for transport of organic arsenic. The energy sources for ArsP function remain to be determined in future studies.

In summary, our study identified a novel transporter that contributes to organic arsenic resistance in *Campylobacter*. To the best of our knowledge, ArsP is the first characterized mechanism for bacterial detoxification of organic arsenic and appears to be quite different in structure and function from previously known transporters for inorganic arsenic, such as ArsB and Acr3. Additionally, ArsP represents the first functionally characterized member in the Duf318 family of transporters. Given that ArsP homologues are present in a number of bacterial and archaeal species and that they are highly conserved in their transmembrane topologies, it is possible that they may play a similar role in the resistance to organic arsenic or similar compounds. Thus, findings in this study may be useful for elucidating functions of ArsP homologues in other organisms. Furthermore, the identified role of ArsP in organic arsenic resistance suggests that it likely contributes to the ecological fitness of *Campylobacter* in poultry and livestock species, in which organic arsenic compounds are commonly used for production. Finally, results from this study and our previous work (9) indicate that the arsenic resistance mechanisms in *Campylobacter* do not confer cross-resistance to clinically used antibiotics. These findings significantly expand our knowledge of antimicrobial resistance in a major food-borne pathogen, reveal a new mechanism for organic arsenic resistance in bacterial organisms, and provide new insights into the adaptive mechanisms of *Campylobacter* in animal reservoirs.

ACKNOWLEDGMENTS

This work is supported by grant number R01DK063008 from the National Institute of Diabetes and Digestive and Kidney Diseases. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the funding agencies.

We thank Bing Wang (Iowa State University) for help in statistical analysis.

REFERENCES

1. **Ruiz-Palacios GM.** 2007. The health burden of *Campylobacter* infection and the impact of antimicrobial resistance: playing chicken. Clin. Infect. Dis. **44:**701–703. http://dx.doi.org/10.1086/509936.
2. **Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM.** 2011. Foodborne illness acquired in the United States—major pathogens. Emerg. Infect. Dis. **17:**7–15. http://dx.doi.org/10.3201/eid1701.09-1101p1.
3. **Humphrey T, O'Brien S, Madsen M.** 2007. Campylobacters as zoonotic pathogens: a food production perspective. Int. J. Food Microbiol. **117:**237–257. http://dx.doi.org/10.1016/j.ijfoodmicro.2007.01.006.
4. **Chapman HD, Johnson ZB.** 2002. Use of antibiotics and roxarsone in broiler chickens in the USA: analysis for the years 1995 to 2000. Poult. Sci. **81:**356–364.
5. **Garbarino JR, Bednar AJ, Rutherford DW, Beyer RS, Wershaw RL.** 2003. Environmental fate of roxarsone in poultry litter. I. Degradation of roxarsone during composting. Environ. Sci. Technol. **37:**1509–1514. http://dx.doi.org/10.1021/es026219q.
6. **Stolz JF, Perera E, Kilonzo B, Kail B, Crable B, Fisher E, Ranganathan M, Wormer L, Basu P.** 2007. Biotransformation of 3-nitro-4-hydroxybenzene arsonic acid (roxarsone) and release of inorganic arsenic by *Clostridium* species. Environ. Sci. Technol. **41:**818–823. http://dx.doi.org/10.1021/es061802i.
7. **Jackson BP, Bertsch PM.** 2001. Determination of arsenic speciation in poultry wastes by IC-ICP-MS. Environ. Sci. Technol. **35:**4868–4873. http://dx.doi.org/10.1021/es0107172.
8. **Rosen BP.** 2002. Biochemistry of arsenic detoxification. FEBS Lett. **529:**86–92. http://dx.doi.org/10.1016/S0014-5793(02)03186-1.
9. **Wang L, Jeon B, Sahin O, Zhang Q.** 2009. Identification of an arsenic resistance and arsenic-sensing system in *Campylobacter jejuni*. Appl. Environ. Microbiol. **75:**5064–5073. http://dx.doi.org/10.1128/AEM.00149-09.
10. **Qin J, Rosen BP, Zhang Y, Wang G, Franke S, Rensing C.** 2006. Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite S-adenosylmethionine methyltransferase. Proc. Natl. Acad. Sci. U. S. A. **103:**2075–2080. http://dx.doi.org/10.1073/pnas.0506836103.
11. **Butcher BG, Deane SM, Rawlings DE.** 2000. The chromosomal arsenic resistance genes of *Thiobacillus ferrooxidans* have an unusual arrangement and confer increased arsenic and antimony resistance to *Escherichia coli*. Appl. Environ. Microbiol. **66:**1826–1833. http://dx.doi.org/10.1128/AEM.66.5.1826-1833.2000.
12. **Butcher BG, Rawlings DE.** 2002. The divergent chromosomal *ars* operon of *Acidithiobacillus ferrooxidans* is regulated by an atypical ArsR protein. Microbiology **148:**3983–3992.
13. **Cai J, Salmon K, DuBow MS.** 1998. A chromosomal *ars* operon homologue of *Pseudomonas aeruginosa* confers increased resistance to arsenic and antimony in *Escherichia coli*. Microbiology **144**(Part 10)**:**2705–2713.
14. **Diorio C, Cai J, Marmor J, Shinder R, DuBow MS.** 1995. An *Escherichia coli* chromosomal *ars* operon homolog is functional in arsenic detoxification and is conserved in gram-negative bacteria. J. Bacteriol. **177:**2050–2056.
15. **López-Maury L, Florencio FJ, Reyes JC.** 2003. Arsenic sensing and resistance system in the cyanobacterium *Synechocystis* sp. strain PCC 6803. J. Bacteriol. **185:**5363–5371. http://dx.doi.org/10.1128/JB.185.18.5363-5371.2003.
16. **Sato T, Kobayashi Y.** 1998. The *ars* operon in the skin element of *Bacillus subtilis* confers resistance to arsenate and arsenite. J. Bacteriol. **180:**1655–1661.
17. **Suzuki K, Wakao N, Kimura T, Sakka K, Ohmiya K.** 1998. Expression and regulation of the arsenic resistance operon of *Acidiphilium multivorum* AIU 301 plasmid pKW301 in *Escherichia coli*. Appl. Environ. Microbiol. **64:**411–418.
18. **Dey S, Rosen BP.** 1995. Dual mode of energy coupling by the oxyanion-translocating ArsB protein. J. Bacteriol. **177:**385–389.
19. **Saltikov CW, Olson BH.** 2002. Homology of *Escherichia coli* R773 *arsA*, arsB, and *arsC* genes in arsenic-resistant bacteria isolated from raw sewage and arsenic-enriched creek waters. Appl. Environ. Microbiol. **68:**280–288. http://dx.doi.org/10.1128/AEM.68.1.280-288.2002.
20. **Ji G, Silver S.** 1992. Reduction of arsenate to arsenite by the ArsC protein of the arsenic resistance operon of *Staphylococcus aureus* plasmid pI258.

Proc. Natl. Acad. Sci. U. S. A. **89:**9474–9478. http://dx.doi.org/10.1073/pnas.89.20.9474.
21. **Ajees AA, Yang J, Rosen BP.** 2011. The ArsD As(III) metallochaperone. Biometals **24:**391–399. http://dx.doi.org/10.1007/s10534-010-9398-x.
22. **Yang J, Rawat S, Stemmler TL, Rosen BP.** 2010. Arsenic binding and transfer by the ArsD As(III) metallochaperone. Biochemistry **49:**3658–3666. http://dx.doi.org/10.1021/bi100026a.
23. **Lin YF, Walmsley AR, Rosen BP.** 2006. An arsenic metallochaperone for an arsenic detoxification pump. Proc. Natl. Acad. Sci. U. S. A. **103:**15617–15622. http://dx.doi.org/10.1073/pnas.0603974103.
24. **Neyt C, Iriarte M, Thi VH, Cornelis GR.** 1997. Virulence and arsenic resistance in yersiniae. J. Bacteriol. **179:**612–619.
25. **Ye J, Yang HC, Rosen BP, Bhattacharjee H.** 2007. Crystal structure of the flavoprotein ArsH from *Sinorhizobium meliloti*. FEBS Lett. **581:**3996–4000. http://dx.doi.org/10.1016/j.febslet.2007.07.039.
26. **Rosenstein R, Nikoleit K, Gotz F.** 1994. Binding of ArsR, the repressor of the *Staphylococcus xylosus* (pSX267) arsenic resistance operon to a sequence with dyad symmetry within the *ars* promoter. Mol. Gen. Genet. **242:**566–572. http://dx.doi.org/10.1007/BF00285280.
27. **Wu J, Rosen BP.** 1991. The ArsR protein is a trans-acting regulatory protein. Mol. Microbiol. **5:**1331–1336. http://dx.doi.org/10.1111/j.1365-2958.1991.tb00779.x.
28. **Zhang YB, Monchy S, Greenberg B, Mergeay M, Gang O, Taghavi S, van der Lelie D.** 2009. ArsR arsenic-resistance regulatory protein from *Cupriavidus metallidurans* CH34. Antonie Van Leeuwenhoek **96:**161–170. http://dx.doi.org/10.1007/s10482-009-9313-z.
29. **Murphy JN, Saltikov CW.** 2009. The ArsR repressor mediates arsenite-dependent regulation of arsenate respiration and detoxification operons of *Shewanella* sp. strain ANA-3. J. Bacteriol. **191:**6722–6731. http://dx.doi.org/10.1128/JB.00801-09.
30. **Papadopoulos JS, Agarwala R.** 2007. COBALT: constraint-based alignment tool for multiple protein sequences. Bioinformatics **23:**1073–1079. http://dx.doi.org/10.1093/bioinformatics/btm076.
31. **Claros MG, von Heijne G.** 1994. TopPred II: an improved software for membrane protein structure predictions. Comput. Appl. Biosci. **10:**685–686.
32. **Nugent T, Jones DT.** 2009. Transmembrane protein topology prediction using support vector machines. BMC Bioinformatics **10:**159. http://dx.doi.org/10.1186/1471-2105-10-159.
33. **Krogh A, Larsson B, von Heijne G, Sonnhammer EL.** 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J. Mol. Biol. **305:**567–580. http://dx.doi.org/10.1006/jmbi.2000.4315.
34. **Spyropoulos IC, Liakopoulos TD, Bagos PG, Hamodrakas SJ.** 2004. TMRPres2D: high quality visual representation of transmembrane protein models. Bioinformatics **20:**3258–3260. http://dx.doi.org/10.1093/bioinformatics/bth358.
35. **Crooks GE, Hon G, Chandonia JM, Brenner SE.** 2004. WebLogo: a sequence logo generator. Genome Res. **14:**1188–1190. http://dx.doi.org/10.1101/gr.849004.
36. **Clinical and Laboratory Standards Institute.** 2006. Performance standards for antimicrobial susceptibility testing; 16th informational supplement. CLSI M100-S16. Clinical and Laboratory Standards Institute, Wayne, PA.
37. **Shen Z, Han J, Wang Y, Sahin O, Zhang Q.** 2013. The contribution of ArsB to arsenic resistance in *Campylobacter jejuni*. PLoS One **8:**e58894. http://dx.doi.org/10.1371/journal.pone.0058894.
38. **Sapkota AR, Price LB, Silbergeld EK, Schwab KJ.** 2006. Arsenic resistance in *Campylobacter* spp. isolated from retail poultry products. Appl. Environ. Microbiol. **72:**3069–3071. http://dx.doi.org/10.1128/AEM.72.4.3069-3071.2006.
39. **Noormohamed A, Fakhr MK.** 2013. Arsenic resistance and prevalence of arsenic resistance genes in *Campylobacter* jejuni and *Campylobacter* coli isolated from retail meats. Int. J. Environ. Res. Public Health **10:**3453–3464. http://dx.doi.org/10.3390/ijerph10083453.
40. **Shi W, Dong J, Scott RA, Ksenzenko MY, Rosen BP.** 1996. The role of arsenic-thiol interactions in metalloregulation of the *ars* operon. J. Biol. Chem. **271:**9291–9297. http://dx.doi.org/10.1074/jbc.271.16.9291.
41. **Yanisch-Perron C, Vieira J, Messing J.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119. http://dx.doi.org/10.1016/0378-1119(85)90120-9.
42. **Yao R, Alm RA, Trust TJ, Guerry P.** 1993. Construction of new *Campylobacter* cloning vectors and a new mutational cat cassette. Gene **130:**127–130. http://dx.doi.org/10.1016/0378-1119(93)90355-7.
43. **Parkhill J, Wren BW, Mungall K, Ketley JM, Churcher C, Basham D, Chillingworth T, Davies RM, Feltwell T, Holroyd S, Jagels K, Karlyshev AV, Moule S, Pallen MJ, Penn CW, Quail MA, Rajandream MA, Rutherford KM, van Vliet AH, Whitehead S, Barrell BG.** 2000. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. Nature **403:**665–668. http://dx.doi.org/10.1038/35001088.