HHS Public Access

Author manuscript

Anaerobe. Author manuscript; available in PMC 2017 June 01.

Published in final edited form as:

Anaerobe. 2016 June ; 39: 117–123. doi:10.1016/j.anaerobe.2016.03.012.

Expression of arsenic resistance genes in the obligate anaerobe *Bacteroides vulgatus* ATCC 8482, a gut microbiome bacterium

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Abstract

The response of the obligate anaerobe *Bacteroides vulgatus* ATCC 8482, a common human gut microbiota, to arsenic was determined. *B. vulgatus* ATCC 8482 is highly resistant to pentavalent As(V) and methylarsenate (MAs(V)). It is somewhat more sensitive to trivalent inorganic As(III) but 100-fold more sensitive to methylarsenite (MAs(III)) than to As(III). *B. vulgatus* ATCC 8482 has eight continuous genes in its genome that we demonstrate form an arsenical-inducible transcriptional unit. The first gene of this *ars* operon, *arsR*, encodes a putative ArsR As(III)-responsive transcriptional repressor. The next three genes encode proteins of unknown function. The remaining genes, *arsDABC*, have well-characterized roles in detoxification of inorganic arsenic, but there are no known genes for MAs(III) resistance. Expression of each gene after exposure to trivalent and pentavalent inorganic and methylarsenicals was analyzed. MAs(III) was the most effective inducer. The *arsD* gene was the most highly expressed of the *ars* operon genes. These results demonstrate that this anaerobic microbiome bacterium has arsenic-responsive genes that confer resistance to inorganic arsenic and may be responsible for the organism's ability to maintain its prevalence in the gut following dietary exposure to inorganic arsenic.

Keywords

Bacteroides vulgatus; Arsenic; Methylarsenic; Anaerobe; Microbiome; Regulation

1. Introduction

Arsenic is the most ubiquitous toxic substance in the environment. It contaminates soil, drinking water and our food supply. Tens of millions of people world-wide are exposed to arsenic by ingestion, inhalation, and dermal absorption routes. In the United States, as many as 25 million people are estimated to drink water with an arsenic level of >10 ppb (parts per billion) [1], the upper limit recommended by the World Health Organization and the maximal containment level allowable by the U.S. Environmental Protection Agency (EPA). The EPA and the Agency for Toxic Substances and Disease Registry (ATSDR) rank arsenic

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Competing interests

None declared.

first on the U.S. Priority List of Hazardous Substances (http://www.atsdr.cdc.gov/SPL/index.html). The U.S. Food and Drug Administration (FDA) considers that arsenic endangers the safety of our food supply (http://www.fda.gov/Food/FoodborneIllnessContaminants/Metals/ucm319870.htm). In countries such as China, arsenic in rice has been associated with increased risk of cancer in exposed populations [2]. Consequently, the United Nations food standards body Codex Alimentarius Commission recommends that the level of arsenic in rice should not exceed 0.2 mg/kg (http://www.fao.org/news/story/en/item/238558/icode/)/. Environmental arsenic is also highly correlated with cardiovascular disease [3], diabetes [4,5], and other human disorders [6].

In addition, humans are exposed to organoarsenicals, which can end up in water and food supplies. Both methylated and aromatic arsenicals have been used for decades for agriculture and animal husbandry. Pentavalent methylarsenate (MAs(V)) has been used as the herbicide monosodium methylarsenate (MSMA) [7]; and is still used in the United States for the treatment of cotton fields, golf courses, turf management and highway medians. Roxarsone (4-hydroxy-3-nitrobenzenearsonic), nitarsone (4-nitrophenyl)arsonic acid and p-ASA(V) (*p*-aminophenyl arsonic acid or Atoxyl) are produced and used today in many countries as antimicrobial growth promoters for poultry and swine to control *Coccidioides* infections [8]. Even though Pfizer has voluntarily suspended U.S. production of roxarsone and atoxyl, they still produce the chemically-related compound nitarsone, the only treatment for blackhead disease, or histomoniasis, in turkeys. These aromatic arsenicals are introduced into the environment when chicken litter is used as fertilizer [8]. Pentavalent organoarsenicals are relatively nontoxic, but the trivalent forms are exceptionally toxic. Aromatic and methylarsenicals are activated to their toxin forms by microbial reduction [9], which can contaminate water supplies though runoffs from farms [10], golf courses [11,12] and other treated soils. Moreover, food crops such as rice accumulate methylated arsenicals, providing an additional source of dietary organoarsenicals [13,14].

The human gut has at least 10^{13} microorganisms, containing 100-fold more genes than our own genome, and more than 90% are anaerobes. The majority of human gut microbiome species fall within four phyla, Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes [15]. In the rat gut microbiome arsenic affects the diversity of microbial populations [16] and their metabolism [17]. Exposure to arsenite disturbs the mouse gut microbiome and substantially alters its metabolomics profile [17]. The prevalence of four families of Firmicutes bacteria significantly decreased, whereas Bacteroidetes families remained relatively unchanged following arsenic exposure, suggesting that *Bacteroides* species may be intrinsically resistant to arsenic. In spite of the impact of arsenic on the microbiome, nothing is known about the effect of arsenic on microbiome gene expression. Our gut microbiome is exposed to dietary arsenic before it enters our blood stream; in effect, it is potentially our first-line for sensing environmental arsenic. There are only a few studies of anaerobic arsenic-inducible gene expression in environmental microbes such as the facultative anaerobe *Shewanella putrefaciens* [18,19] and, to our knowledge, none in the obligate anaerobes of the gut microbiome. This lack of information limits our understanding of how the microbiome responds to the dietary arsenic.

Members of the phylum Bacteroidetes form the major bacterial species in the human gut microbiome [20]. One highly abundant microbiome species is the obligate anaerobe *Bacteroides vulgatus* [21], Most bacteria and archaea have arsenic resistance (*ars*) operons responsible for a wide variety of arsenic biotransformations [22]. *Bacteroides vulgatus* ATCC 8482 is a Gram-negative obligate anaerobe originally isolated in 1933 from human feces [23]. From its genome sequence, it has a putative *ars* operon with eight genes. The first gene encodes a putative ArsR As(III)-responsive transcription repressor that might control expression of the operon. The physiological functions of the next three putative gene products are unknown. The last four correspond to the well-characterized *arsDABC* genes [24]. ArsD is an arsenic metallochaperone that donates As(III) to the ArsAB ATPase, an As(III) efflux pump. ArsC is an arsenate reductase that transforms As(V) into the As(III), the substrate of the efflux pump. It is interesting that an obligate anaerobe would have the gene for an enzyme that reduces pentavalent inorganic arsenic, implying that arsenate might be able to reach the lower intestine in the oxidized form.

In this study expression of each of the *ars* genes was determined by quantitative real time polymerase chain reaction (Q-RT-PCR) in response to exposure to trivalent arsenite, pentavalent arsenate and to the methylated arsenicals MAs(V) and MAs(III). The *ars* genes were induced most effectively by trivalent arsenicals. The *arsD* gene was the most highly expressed of the *ars* operon genes. Since these genes might be expressed even before arsenic enters our blood stream, we propose that expression of the *ars* genes in a gut microbe has to potential to be adapted for use as a biomarker of dietary exposure to arsenic. Knowledge of the response of a gut microbe to arsenic exposure increases understanding of how microbiome diversity is maintained.

2. Materials and methods

2.1. Anaerobic growth of Bacteroides vulgatus ATCC 8482

Bacteroides vulgatus Eggerth and Gagnon, ATCC 8482 (NCTC 11154) was purchased from the American Type Culture Collection (Manassas, VA). To revive the strains, cells were initially grown on 1.5% agar plates consisting of tryptic soy with 5% rabbit blood (Fisher Scientific, Pittsburgh, PA) in an anaerobic jar with GasPak™ for 36 h at 37 °C. Cultures were then grown in the anaerobic jar in degassed Schneider's insect medium containing 20% heat-inactivated fetal bovine serum (Sigma-Aldrich, St. Louis, MO) for 24 h at 37 °C with shaking.

2.2. Arsenic resistance assays

Cultures of *B. vulgatus* ATCC 8482 were grown at 37 °C with shaking in degassed Schneider's insect medium containing 20% heat-inactivated fetal bovine serum containing the indicated concentrations of As(V), As(III), MAs(V) or MAs(III) in an anaerobic jar with GasPak™ After approximately 24 h, when the cultures had attained an A_{600nm} = 1.0, the cells were collected at 4 °C by centrifugation in tightly capped tubes filled with nitrogen gas.

2.3. Isolation of nucleic acids

Genomic DNA was isolated as described by Liao et al. (2011) using a QIAamp DNA Mini Kit (QIAGEN, Valencia, CA). Total RNA from *B. vulgatus* was isolated using TRIZOL reagent (Life Sciences, ThermoFisher Scientific, Grand Island, NY) according to the manufacturer's protocol. DNA was removed from total RNA preparation using TURBO DNAfree Kit (Ambion, ThermoFisher Scientific, Grand Island, NY) according to the manufacturer's instructions. RNA integrity was confirmed by denaturing agarose gel electrophoresis.

2.4. Reverse transcription (RT) and quantitative real-time (Q-RT) polymerase chain reaction (PCR)

cDNA synthesis was carried out with 0.5 μg of total RNA using an AccuScript High Fidelity 1st strand cDNA synthesis kit (Agilent, Santa Clara, CA) according to the manufacturer's instructions. The reactions were incubated at 25 °C for 10 min and 42 °C for 30 min, followed by heat inactivation at 95 °C for 5 min. The reaction mix was then neutralized with sodium acetate and purified using a Qiagen PCR purification kit according to the manufacturer's instructions.

For RT-PCR total RNA isolated from exponentially growing *B. vulgatus* ATCC 8482 in the absence or presence of 13 μM MAs(III) was used as template in a reverse transcriptase reaction to generate cDNAs that were then amplified with the indicated primer sets (Table 1). The primers covering all eight genes with overlap between them were purchased from Integrated DNA technologies (IDT) (Coralville, lA). cDNA of 16S rRNA was used as control to normalize gene expression. Genomic DNA of *B. vulgatus* ATCC 8482 was used as positive control for all primers amplicons. PCR products were resolved on 1% agarose gels using standard methods [25] and stained with ethidium bromide.

For Q-RT-PCR, 2 μl of diluted purified cDNA reaction corresponding to 6 ng of template RNA, were used in a 10 μl reaction containing forward and reverse primers for the target genes (Table 2) and 1× iQSYBR Green supermix (Bio-Rad Laboratories, Inc., Hercules, CA). Primer sets were designed using IDT's Primer-Quest software and only efficiency between 98% and 102% primers were used in the current study. qPCR reactions were analyzed using an Eppendorf Realplex2 PCR instrument with the following thermal cycling conditions: initial denaturation at 95 °C for 3 min followed by 40 cycles of 95 °C for 15 s and 65 °C for 20 s. A final melting curve analysis was performed for each reaction to confirm that the PCR generated a single amplification product. The relative abundance of target amplicons between samples was estimated using 16S rRNA, *pfk* or σ^{70} as controls by the $2^{-\Delta\Delta CT}$ method [26]. Error bars were calculated from the mean ± SD of three independent experiments in triplicate.

3. Results

3.1. Anaerobic resistance to inorganic and organic arsenicals in B. vulgatus ATCC 8482

Nearly every microbe has *ars* operons that confer resistance to arsenicals, both inorganic and organic [22]. The level of resistance is quite variable. Some aerobic microorganisms are

sensitive to micromolar concentrations of highly toxic trivalent aromatic arsenicals [27], while others are resistant to nearly molar concentrations of pentavalent arsenate [28]. There have been few studies of arsenic resistance in anaerobes, and, to our knowledge, none in inhabitants of the gut microbiome. Resistance to As(III), As(V), MAs(III) and MAs(V) on anaerobic growth *B. vulgatus* ATCC 8482 in pure culture was examined (Fig. 1). *B. vulgatus* ATCC 8482 is quite resistant to the pentavalent species arsenate and methylarsenate, with growth in medium with 10–100 mM. The cells are somewhat more sensitive to trivalent arsenite but still grow at millimolar concentrations. Trivalent methylarsenite is the most toxic, with complete growth inhibition at 100 μM MAs(III). It should be pointed out that the free concentration of arsenicals was probably less than the added concentration since the cells were grown in very rich Schneider's insect medium with 20% fetal bovine serum, which, like most complex media, has many thiol-containing molecules that may bind arsenicals. The relative tolerance to arsenate and arsenite implies the presence of arsenic resistance genes in *B. vulgatus* ATCC 8482.

3.2. The ars operon of B. vulgatus ATCC 8482

The complete sequence of the *B. vulgatus* ATCC 8482 includes eight contiguous genes that might compose an *ars* operon (Fig. 2A). The first product encodes a putative 108-residue ArsR As(III)-responsive transcriptional repressor (accession number ABR41782.1). Three different As(III) binding sites, each of which is composed of three cysteine residues located in different regions of the protein, have been identified in ArsRs [29–31]. BvArsR does not have any of the three known As(III)-binding motifs, so it cannot be assumed that it is in fact an As(III)-responsive repressor. It has five cysteine residues, three of which (Cys99, Cys106 and Cys107) near the C-terminus might form a new ArsR As(III) binding site. The next gene, *orf1*, encodes a typical 77-residue thioredoxin (accession number ABR41783.1). While its role in arsenic resistance is not known, it could serve as the source of reducing potential for both the *orf3* and *arsC* gene products, as described below. The next gene, *orf2*, potentially encodes a 147-residue protein (accession number ABR41784.1) annotated as a thioredoxin-like protein. The *orf3* gene product (accession number ABR41785.1) is annotated as a putative cytochrome c biogenesis protein and has similarities to the disulfide bond isomerase DsbD, which uses thioredoxin to reduce disulfide bonds in periplasmic proteins [32]. The role of the Orf2 and Orf3 proteins in arsenic resistance is unknown, but it is reasonable to assume that they are in an *ars* operon because they have an arsenic-related function. The product of the next gene is a 108-residue ArsD metallochaperone (accession number ABR41786.1) [33] that ferries intracellular As(III) to the next gene product, the 570-residues ArsA ATPase (accession number ABR41787.1) [34,35]. The next gene encodes the 142-residue ArsC arsenate reductase (accession number ABR41788.1) that couples the reducing potential of thioredoxin to conversion of As(V) to As(IIII), the substrate of the product of the eighth gene, the 348-residue Acr3 efflux permease (accession number ABR41789.1) [36,37]. Acr3 can also form a complex with ArsA to form an As(III)-translocating efflux pump [38].

Since the putative *arsR* gene does not have a known As(III) binding motif, and the three genes following *arsR* cannot be assigned a role in arsenic detoxification, it is not clear if this cluster of eight genes comprise an *ars* operon. To examine this question, co-transcription of

the genes after induction by MAs(III) was examined by reverse transcription polymerase chain reaction (RT-PCR). A series of oligonucleotide primers covering all eight genes with overlap between them was designed for reverse transcription polymerase chain reaction (RT-PCR) (Fig. 2B and Table 1). Cells of *B. vulgatus* ATCC 8482 were grown anaerobically in the presence or absence of MAs(III) as inducer. Total RNA was isolated and used as template for reverse transcription of cDNAs in the absence (Fig. 2C, gel 1) or presence (gel 2) of 13 μM (1 ppm) MAs(III). In the absence of inducer no *ars* genes were expressed (gel 1). In the presence of inducer, each gene was amplified using primers that originated from the adjacent gene (gel 2), confirming co-transcription of all eight genes. The level of transcription of the housekeeping gene for 16S rRNA with or without inducer was used to normalize expression of the *ars* genes (lane 10 in each gel). To demonstrate the specificity of the primers, genomic DNA was used in place of cDNA (gel 3). These results demonstrate that the eight genes comprise a single *ars* operon.

3.3. Induction of the B. vulgatus ATCC 8482 ars operon

In aerobic microorganisms, *ars* operons sense trivalent inorganic and organic arsenicals [27]. Pentavalent arsenicals do not directly induce but must first be reduced. However, to our knowledge, there have been no studies of the transcription of *ars* genes anaerobic microbiome bacteria in response to exposure to inorganic arsenic or organic arsenicals, nor has reduction of MAs(III) been demonstrated in an anaerobe.

Our research shows that transcription of the four *ars* genes of known function responds to addition of As(III) (Fig. 3A), As(V) (Fig. 3B) and MAs(III) (Fig. 3C) to pure cultures of *B. vulgatus* ATCC 8482. Induction with 10 ppm (0.13 mM) As(III) was about 4–8 fold relative to 16S rRNA expression (to correspond with the EPA's usage, the arsenic concentrations in Fig. 3 are expressed as ppm). The same level of induction required 1000-fold more As(V). Differences in the rates of uptake of the arsenicals could contribute to the observed differential expression, but a more likely explanation is that the true inducers are trivalent, not pentavalent, arsenicals, and As(V) must be reduced to As(III) in order to induce. MAs(III) was by far the best inducer. It induced the *ars* genes 200–600 fold relative to expression of 16S rRNA at 1000-fold lower concentration than either inorganic arsenate or arsenite. The *arsA, acr3* and *arsC* genes were each expressed at nearly the same levels as each other, no matter which arsenical was used as inducer, but the *arsD* gene was consistently expressed at 2–4 fold higher levels than the others. The three *orfs* of unknown function are also induced by MAs(III) using expression of 16S rRNA, *pfk* (6-phosphofructokinase) or σ^{70} as housekeeping genes (Fig. 4). The *orf1* gene, which encodes a putative thioredoxin, was transcribed nearly as well as *arsD*. The *orf3* gene was transcribed at about half that level, and *orf2* was transcribed at about 10–15% of *arsD*. Segmental differences in mRNA stability within the polycistronic transcript of another *ars* operon was proposed to produce differential gene expression [39] and may explain these results.

4. Discussion

Species within the *Bacteroides* genus, including *B. vulgatus*, colonize the intestinal tract of humans commensally. They increase nutrient utilization by their hosts through

biodegradation of complex polysaccharides into more easily digested simpler sugars [40]. A decrease in the proportion of Bacteroidetes in the microbiome relative to Firmicutes is associated with obesity, and weight loss is associated with an increase in the ratio of Bacteroidetes to Firmicutes [41,42]. In mice exposure to inorganic arsenic in drinking water produces dysbiosis, with an increase in the relative proportion of members of the Bacteroidetes phylum relative to Firmicutes, leading to a perturbation in metabolite profiles [17]. The change in the gut microbiome composition is due a decrease in Firmicutes, while Bacteroidetes remain relatively constant during arsenic exposure. Here we demonstrate that *B. vulgatus* ATCC 8482 is highly resistant to inorganic arsenic, most likely the result of expression of the genes of chromosomal *ars* operon. In contrast, neither *Clostridium difficile* (Accession number NC_009089) nor *Lactobacillus gasseri* (Accession number NC_008530.1), both members of the Firmicutes phylum present in the human gut microbiome, contains an *ars* operon in its genome. We propose that the presence of *ars* operons in Bacteroidetes and their absence in Firmicutes leads to increased fitness of Bacteroidetes relative to Firmicutes.

A distinctive feature of *B. vulgatus ars* gene expression is that MAs(III) is by far the best inducer. Compared with As(III), MAs(III) induced the *arsD* gene to about 50-fold higher levels at three orders of magnitude lower concentration. Strikingly, *B. vulgatus* ATCC 8482 is also nearly 100-fold more sensitive to MAs(III) than to As(III) (Fig. 1). This difference may be due to some degree to the higher toxicity of MAs(III) compared to As(III). However, none of the genes in the *ars* operon correspond to any of the known MAs(III) resistance genes for MAs(III): the ArsI C–As lyase [43], the ArsH MAs(III) oxidase [44] or the ArsP MAs(III) efflux permease [45], so extreme MAs(III) sensitivity is not unexpected. ArsI and ArsH require oxygen, and their genes do not appear to be present in the anaerobic bacteria of the gut microbiome. Yet, humans are exposed to MAs(III) in run-offs from pesticide-treated farms, golf courses and other soils. Rice accumulates methylated arsenicals and can also be a source of dietary exposure. The major source of MAs(III), however, may be microbiome organisms that methylate arsenic. For example, there are human gut microbiome bacteria that have an *arsM* gene encoding an As(III) *S*-adenosylmethionine methyltransferase, including *Bacteroides fragilis* 3_1_12 (Accession number EFR52264), *Parabacteroides johnsonii* DSM 18315 (Accession number EEC94541) and *Parabacteroides goldsteinii* (Accession number WP_009860065.1). Thus gut microbes could methylate dietary As(III) to MAs(III), which would then induce *Bacteroides* species to higher levels of expression, resulting in high-level resistance of these species in the gut microbiome. This would, in effect, be an autoamplification pathway that could augment the constancy of Bacteroidetes following inorganic arsenic exposure.

While these experiments utilize a single anaerobic *Bacteroides* species grown in pure culture, the data establish a proof of concept that the *ars* genes of this gut microbe are induced by both inorganic As(III) and organic MAs(III). Future experiments will examine expression of *ars* genes in the microbiome.

5. Conclusions

Here we show that *B. vulgatus* ATCC 8482 is very sensitive to the trivalent organoarsenicals methylarsenite, less sensitive to inorganic trivalent As(III) and much less sensitive to pentavalent arsenicals. We analyzed the eight gene *ars* operon of *B. vulgatus* ATCC 8482. This arsenic-inducible transcriptional unit confers resistance to trivalent arsenicals, with MAs(III) being the most effective inducer, followed by As(III). Our results imply that this gut microbiome organism maintains an *ars* operon as the result of dietary exposure to inorganic arsenic. The selectivity for MAs(III) implies that the organism is also exposed to organoarsenicals.

Acknowledgments

This work was supported by the National Institutes of Health grants R37 GM55425 and R01 ES023779. We thank Dr. Rita Mukhopadhyay for advice.

Abbreviations

MAs(III)	Methylarsenite
MAs(V)	methylarsenate
DMAs(V)	dimethylarsenate
MSMA	monosodium methylarsenate
RT-PCR	reverse transcription polymerase chain reaction
Q-RT-PCR	quantitative real-time polymerase chain reaction

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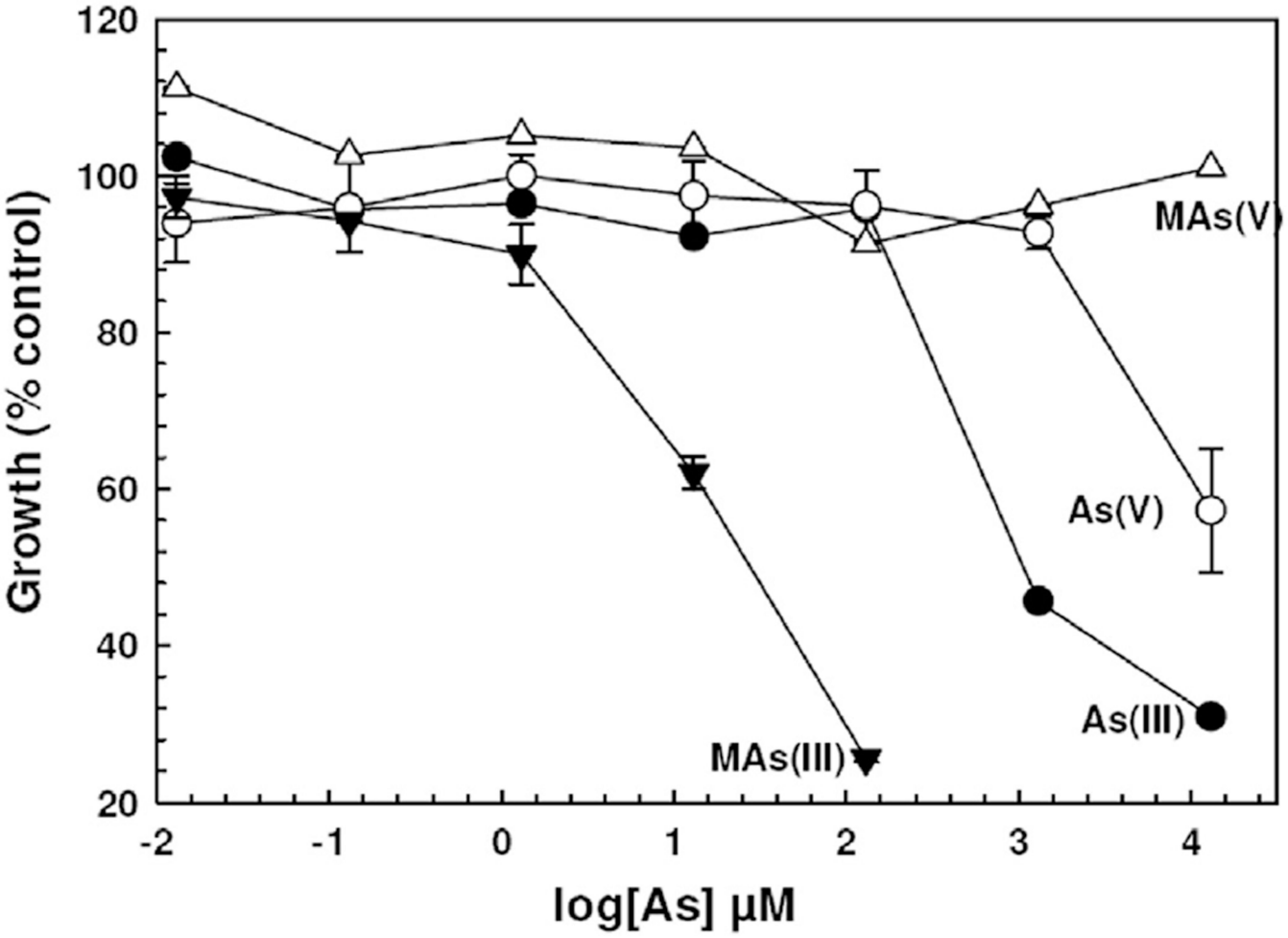

Fig. 1. Resistance to inorganic and organic arsenicals in anaerobic growth *B. vulgatus* strain ATCC 8482

Cultures were grown anaerobically in Schneider's insect medium containing 20% heat-inactivated fetal bovine serum containing the indicated concentrations of As(III) (●), As(V) (o), MAs(III) (▼) or MAs(V) (Δ). After 24 h at 37 °C with shaking, the A_{600nm} was measured. Data are the mean ± SE (n = 3).

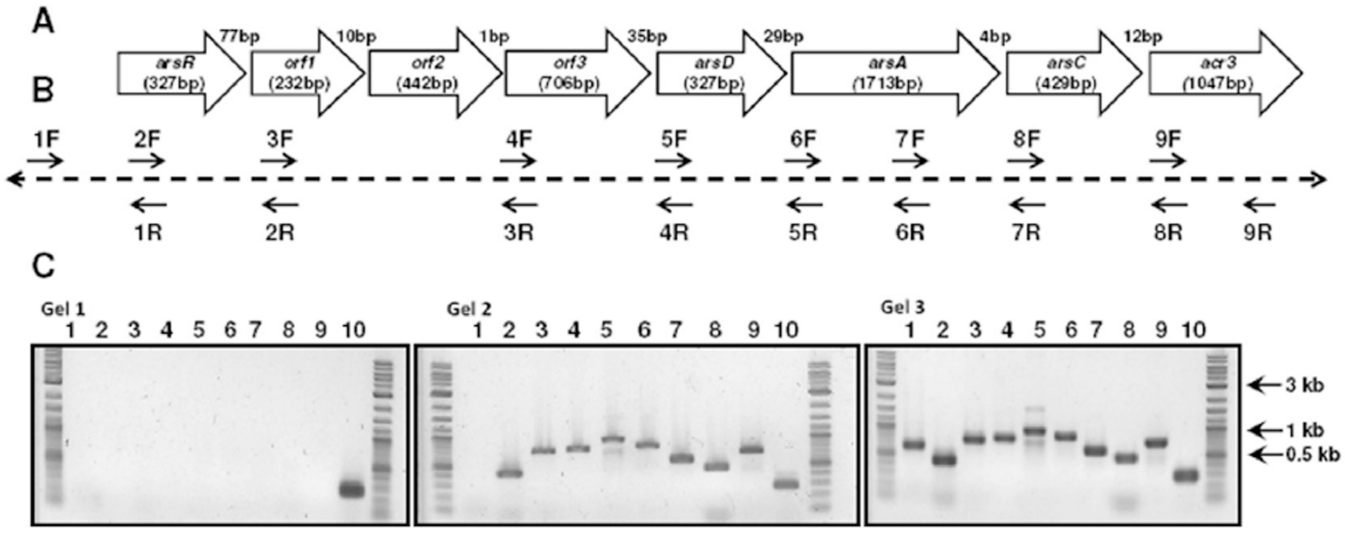

Fig. 2. The *ars* operon of *B. vulgatus* strain ATCC 8482

A. The putative *ars* operon. From the complete genome *Bacteroides vulgatus* strain ATCC 8482 (Accession number CP000139) eight continuous genes encoding a putative *ars* operon were identified. **B. The *ars* transcriptional unit**. Shown are the location of the primers for RT-PCR analysis (Table 1) of the *ars* transcriptional unit relative to the eight putative *ars* genes. **C. RT-PCR analysis**. Total RNA isolated from *B. vulgatus* strain ATCC 8482 in the exponential phase (A_{600nm} = 1.2) in the absence (gel 2) or presence (gel 3) of 1 ppm (13 μM) MAs(III) was used as the template in a reverse transcriptase reaction to generate cDNA and then amplified with the indicated primers. PCR products were resolved on a 1% agarose gel stained with ethidium bromide. Genomic DNA from *B. vulgatus* was used as a positive control for each primer set (gel 1). In each gel lane 10 is the 16S rRNA (Accession number NC_009614.1) PCR product.

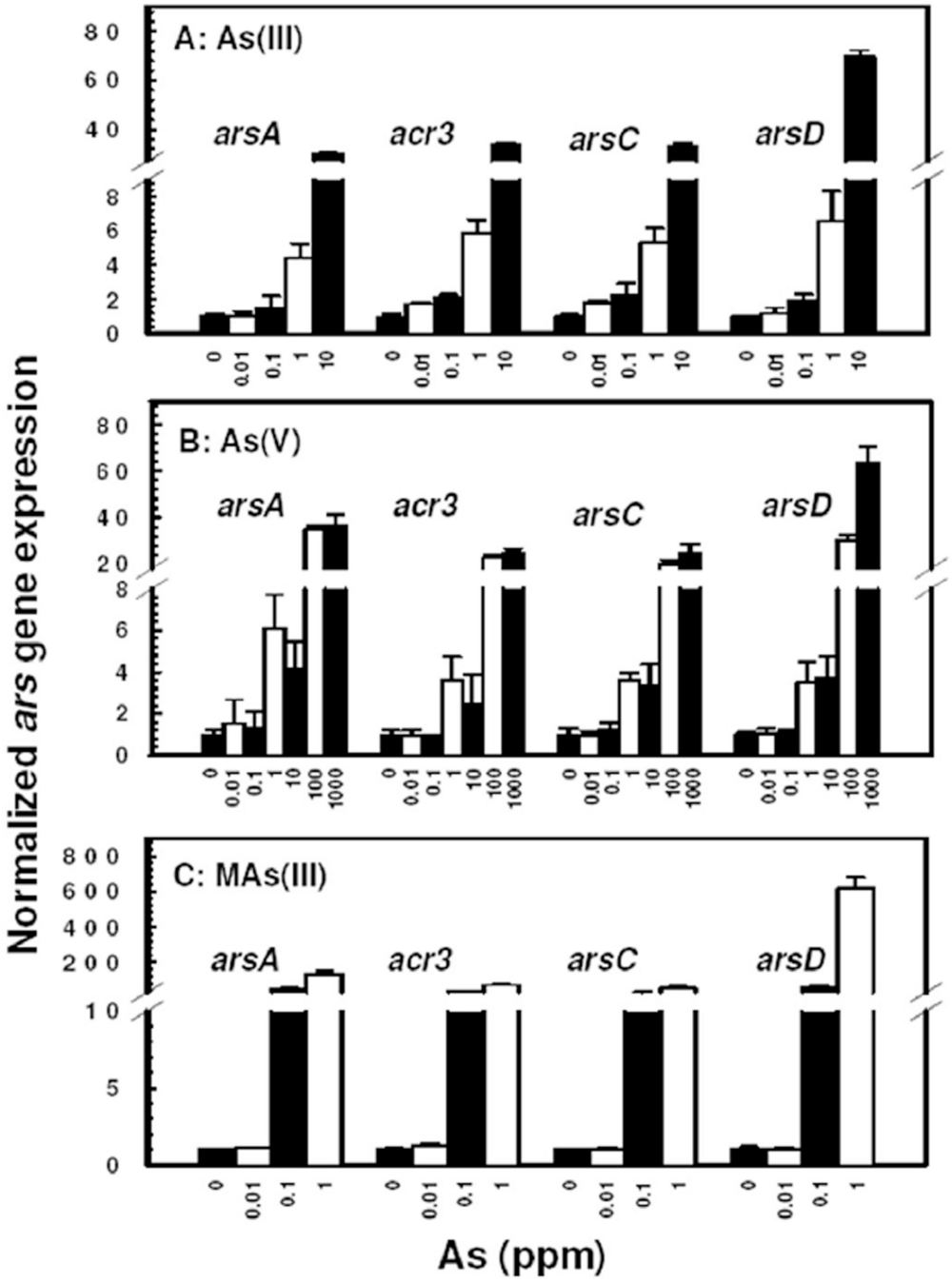

Fig. 3. Transcriptional analysis of the *arsA, acr3, arsC and arsD* genes

Anaerobic cultures of *B. vulgatus* strain ATCC 8482 were induced with the indicated concentrations in ppm of As(III) (**A**), As(V) (**B**) or MAs(III) (**C**). Total RNA was isolated, and mRNA expression of the *arsA, acr3, arsC* and *arsD* genes were analyzed with the primer sets in Table 2 using the $2^{-\Delta\Delta Ct}$ method [26] normalized to 16S rRNA gene expression. Data are the mean ± SE (n = 3).

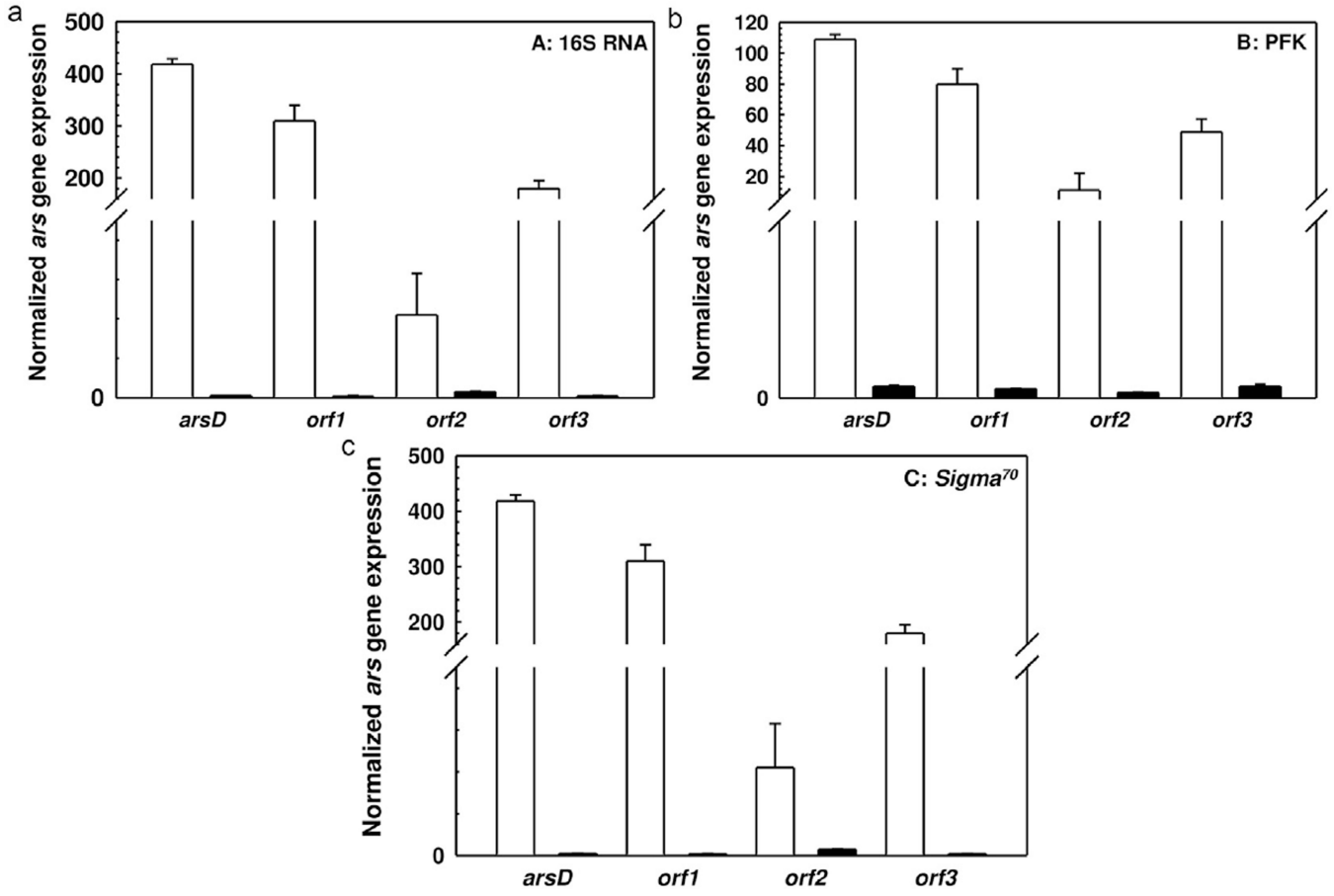

Fig. 4. Transcriptional analysis of *orf1, orf2* and *orf3*

Total RNA was isolated from cultures of *B. vulgatus* strain ATCC 8482 induced with (white bars) or without (black bars) 1 ppm (13 μM) MAs(III) and analyzed as described in the legend to Fig. 3. The *E. coli* housekeeping genes used to normalize expression were A) 16S rRNA B) 6-phosphfructokinase (PFK) (Accession number NC_009614.1) C) σ^{70} (Accession number NC_009614.1). Data are the mean ± SE (n = 3).

Table 1

Oligonucleotide primers for RT-PCR

For determination of operon structure the indicated pairs of forward and reverse RT-PCR primers were used for amplification of overlapping regions of the operon. The dashes before or after the target gene indicate that the primers began or ended outside of the gene.

RT-PCR primer set	Target	Primer sequences	Amplicon length (bp)
1	*-arsR*	Sense: 5′- TACTACACTGGCAGAAGGATTG -3′	617
		Antisense: 5′- AATCTGTTCCTGCTCTACTGTG -3	
2	*arsR-rf1*	Sense: 5′- CACAGTAGAGCAGGAACAGATTGC -3′	422
		Antisense: 5′- CTTGAACACCCGGTTCCTAATAC -3′	
3	*orf1-orf3*	Sense: 5′- TATTAGGAACCGGGTGTTCAAG -3′	723
		Antisense: 5′- TAATAGCGGGAACAGAACTGC -3′	
4	*orf3-arsD*	Sense: 5′- CAGTTCTGTTCCCGCTATTAC -3′	774
		Antisense: 5′- TGTTCCACAAAGACCCGTAG -3′	
5	*arsD-arsA*	Sense: 5′- CTACGGGTCTTTGTGGAACA -3′	948
		Antisense: 5′- GGGACGACTTACCAACACTAAG -3′	
6	*-arsA-*	Sense: 5′- TTGGTAAGTCGTCCCGAAATC -3′	830
		Antisense: 5′- CTTACCGAAGCAGGAGAATCG -3′	
7	*arsA-arsC*	Sense: 5′- CGATTCTCCTGCTTCGGTAAG -3′	518
		Antisense: 5′- ACTTCACCAGTAAACATAGGACAG -3′	
8	*arsC-acr3*	Sense: 5′- CTGTCCTATGTTTACTGGTGAAGT -3′	484
		Antisense: 5′- CTATCCCGAACATGGTAAACGG -3′	
9	*-acr3-*	Sense: 5′- CCGTTTACCATGTTCGGGATAG -3′	703
		Antisense: 5′- ACTTCAACCAGCACACCTAC -3′	
10	−16s rRNA-	Sense: 5′- GGGATGCGTTCCATTAGATAGT -3′	309
		Antisense: 5′- CGGAGTTAGCCGATCCTTATTC -3′	

Table 2

Oligonucleotide primers for Q-RT-PCR

Expression of each gene was determined using the indicated Q-RT-PCR primers.

Q-RT-PCR primer set	Target	Primer sequences	Amplicon length (bp)
11	*orf1*	Sense: 5′- GCTGCGATGCAACACTAATC -3′	78
		Antisense: 5′- CCAAAGCCGGAAGACCTAAA -3′	
12	*orf2*	Sense: 5′- GATTGCGATGCTGGTCTTAATG -3′	92
		Antisense: 5′- CAATGCGGTTAGGCTGTATTTC -3′	
13	*orf2*	Sense: 5′- AAACAGGCGGCTATCTCTTAC -3′	99
		Antisense: 5′- CCAGTCCGGCAACACTATAA -3′	
14	*arsA*	Sense: 5′- CTTGCAGTTCAGGCAGGTATTA -3′	101
		Antisense: 5′- GTCATTGTTTCCGCAGCTTTAC -3′	
15	*acr3*	Sense: 5′- GCAAACGTCCGAGAGGAATAA -3′	77
		Antisense: 5′- AGCTATCCCGAACATGGTAAAC -3′	
16	*arsC*	Sense: 5′- GGGACTATGTTATTACGGTCTGC -3′	98
		Antisense: 5′- TGGGTCGTCAAACCCAATATG -3′	
17	*arsD*	Sense: 5′- ACGTAAGCAATAAGACGGTGAA -3′	112
		Antisense: 5′- TTGGTGGTAGGATAGGTTTGTG -3′	
18	16s rRNA	Sense: 5′- GACTGCCCTATGGGTTGTAAA -3′	98
		Antisense: 5′- CGGAGTTAGCCGATCCTTATTC -3	
19	*6-pfk*	Sense: 5′- CGCCGAATATATAGCCCAAGAA -3′	111
		Antisense: 5′- AGATTACGGTCGTAGGGAGTAG -3′	
20	σ^{70}	Sense: 5′- CTATCTAAAGCACGAGGAGGTAAA -3′	92
		Antisense: 5′- CCAAGGCAGACATACGGATAG -3′	