The *Chromobacterium violaceum* ArsR Arsenite Repressor Exerts Tighter Control on Its Cognate Promoter Than the *Escherichia coli* System

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Environmental bacteria are endowed with several regulatory systems that have potential applications in biotechnology. In this report, we characterize the arsenic biosensing features of the *ars* response system from *Chromobacterium violaceum* in the heterologous host *Escherichia coli*. We show that the native *Pars/arsR* system of *C. violaceum* outperforms the chromosomal *ars* copy of *E. coli* when exposed to micromolar concentrations of arsenite. To understand the molecular basis of this phenomenon, we analyzed the interaction between ArsR regulators and their promoter target sites as well as induction of the system at saturating concentrations of the regulators. *In vivo* titration experiments indicate that ArsR from *C. violaceum* has stronger binding affinity for its target promoter than the regulator from *E. coli* does. Additionally, arsenite induction experiments at saturating regulator concentration demonstrate that although the *Pars/arsR* system from *E. coli* displays a gradual response to increasing concentration of the inducer, the system from *C. violaceum* has a steeper response with a stronger promoter induction after a given arsenite threshold. Taken together, these data demonstrate the characterization of a novel arsenic response element from an environmental bacterium with potentially enhanced performance that could be further explored for the construction of an arsenic biosensor.

**Keywords:** regulatory network, arsenic response system, *cis*-regulatory elements, *ars* operon, ArsR/SmtB family

**INTRODUCTION**

Bacteria that thrive in environments contaminated with toxic compounds are usually endowed with diverse molecular mechanisms related to tolerance to these chemicals (Top and Springael, 2003; Permina et al., 2006). From a physiological point of view, mechanisms for resistance to stressors are usually energy dependent and should be tightly regulated in order to avoid wasting valuable resources when stressors are absent (Nojiri et al., 2004; Diaz et al., 2013). Many bacteria have evolved molecular mechanisms to handle exposure to toxic metals and metalloids. The genes encoding such systems are usually transcriptionally controlled by a plethora of regulatory systems that coordinate gene expression in response to the presence of the cognate chemical.
Several regulatory systems dedicated to the sensing of toxic metals and metalloids have been characterized in bacteria (Busenlehner et al., 2003; Kaur et al., 2006; Permina et al., 2006; Reyes-Caballero et al., 2011). Genomic studies have demonstrated that most of these systems are broadly distributed among different bacterial phyla (Paez-Espino et al., 2009). For instance, transcription factors belonging to the SmtB/ArsR family have been expensively characterized for their role in sensing and controlling gene expression in response to divalent metals (e.g., zinc, nickel, and cadmium) or toxic metalloids (e.g., arsenic and antimonite) (Busenlehner et al., 2003; Eicken et al., 2003; Qin et al., 2007). Members of this family are usually small (~100 aa) regulatory proteins that repress gene expression by blocking access of RNA polymerase to target promoters in the absence of the cognate inducer (VanZile et al., 2000; Kar et al., 2001; Morita et al., 2001; Cavet et al., 2002). These proteins usually act as dimers that bind to target DNA sequences in the apo form. Once in complex with their specific target metal/metalloid, the regulators strongly decrease their affinity for DNA allowing dissociation from the promoter and subsequently, gene expression activation (Kar et al., 1997; Morita et al., 2002, 2003; Eicken et al., 2003; Chauhan et al., 2009).

Understanding the molecular mechanisms behind the transcriptional response to toxic metals and metalloids in bacteria has led to a growing interest in repurposing these systems to construct biosensors for detection of chemicals in the environment (Stocker et al., 2003; Siddiki et al., 2011; Cortés-Salazar et al., 2013; Chen et al., 2014). Such efforts have been made for the detection of arsenic, a highly abundant and extremely toxic metalloid released to the environment as a result of anthropogenic activity (Matschullat, 2000; Mandal and Suzuki, 2002). In comparison to analytical chemistry methods, biosensors (which encompass a biological sensing component and easily detectable output) would provide reliable, specific, and an inexpensive means for the in situ detection of target compounds in environmental samples (Baemunner, 2003).

Constructed biosensors for environmental purposes have generally coupled well-characterized components (usually from bacteria) that are responsive to the target compound with a reporter gene that gives rise to a colorimetric, luminescent, or fluorescent output. In this sense, developed biosensors proved to be useful tools for arsenite and arsenate detection in groundwater (Siegfried et al., 2012) and river water (Siegfried et al., 2015) as a low cost, suitable and transportable alternative to detect the metalloid to prevent or diminish arsenic exposure. Furthermore, the advent of biological circuit design approaches in the field of synthetic biology has allowed the re-wiring of basic molecular components (regulators, DNA binding sites, and operators), which can be reinserted into the host cell to give rise to sensors with enhanced performance (Stocker et al., 2003; Trang et al., 2005; Fernandez et al., 2016; Merulla and van der Meer, 2016). In fact, the addition of ArsR operator downstream of its target promoter generates reduction in the background noise, which reduces the detection limits to as low as one microgram per liter (Merulla and van der Meer, 2016).

Although changing the circuit design can improve the efficiency of the biosensor, the utilization of molecular components with intrinsically enhanced transcriptional performance when induced with the target compound could lead to a system with superior behavior. With this reasoning in mind, we aimed to identify a natural system that displayed enhanced transcriptional response to arsenite (AsIII). We focused on the ars system from Chromobacterium violaceum, a gram-negative bacterium with a low arsenic tolerance level but endowed with a fully functional Pars and ArsR regulatory system (Azevedo et al., 2008; Silva-Rocha et al., 2013a). We demonstrate that the ars system from C. violaceum has superior arsenic induction performance when compared to the chromosomal prototype system in Escherichia coli and that these differences can be traced to the binding affinity of ArsR regulators to their DNA targets and to the occurrence of a stronger transcriptional response under inducing conditions in the C. violaceum system. Taken together, the results shown here demonstrate the potential of environmental bacteria as a reservoir of molecular components with enhanced performance for biosensor design, as well as a characterization of a novel arsenic ArsR transcription factor in bacteria.

**MATERIALS AND METHODS**

**Bacterial Strains, Plasmids, and Growth Conditions**

Bacterial strains, plasmids, and primers used in this study are listed in **Table 1**. E. coli DH5α cells were used for cloning procedures. E. coli W3110 was used as the wild type strain, whereas E. coli AW3110 (Δars operon) was used as the mutant host for testing the circuits. E. coli strains were grown at 37°C in LB media (Sambrook et al., 1989) or M9 minimal media (6.4 g/L Na2HPO4, 1.5 g/L KH2PO4, 0.25 g/L NaCl, and 0.5 g/L NH4Cl) supplemented with 2 mM MgSO4, 0.1 mM casamino acid, and 1% glycerol as the sole carbon source. When required, kanamycin (Km, 50 μg/mL) or chloramphenicol (34 μg/mL) was added to the media to ensure plasmid retention. When cells were grown in minimal media, antibiotics were used at half concentrations. For induction experiments, benzoic acid (Sigma–Aldrich, St. Louis, MO, USA) and sodium arsenite (Sigma–Aldrich) were used at different concentrations.

**Plasmid and Strain Construction**

For analysis of the ars system under negative feedback, DNA fragments containing the Pars/arSR elements from C. violaceum and E. coli were PCR amplified from genomic DNA using primers 5arscvEco/3arscvBam and 5arsEcO/3arsEcBam, respectively (Table 1). DNA fragments were amplified using Phusion High-Fidelity DNA polymerase (New England Biolabs, Ipswich, MA, USA) according to the manufacturer’s protocol. The resulting DNA fragments were cloned into the pMR1 reporter vector (Guazzaroni and Silva-Rocha, 2014), which carries the gfpλva, a short lived variant of GFP. The resulting recombinant plasmids were named pMR1-Parscv::ArSRcv.
pMR1-Parcvi::arsRcvi (Table 1; Supplementary Material). The correct DNA sequence was verified by sequencing using the dideoxy terminal method. Next, the recombinant plasmids were introduced into E. coli W3110 using chemically competent cells (Sambrook et al., 1989). The resulting reporter strains were used for induction experiments. For cloning and analysis of individual promoters, Parcvi and Parscvi were PCR amplified using primers 5arscviEco/3ParscviBam and 5arsecEco/3ParsecBam, gel purified, and cloned into pRV2 using EcoRI/BamHI restriction sites. The resulting plasmids, pRV2-Parcvi and pRV2-Parscvi, were transformed into E. coli W3110 (wild type) and E. coli AW3110 (mutant) strains. To construct the uncoupled circuit, the arsR genes from each organism were cloned under the control of the XylS/Pm system, which is inducible by benzoate (Blatny et al., 1997). Each regulator was amplified using primers introduced a strong RBS sequence at the 5′ end and pSEVA438 inserted with the operator, promoter and regulation region as native arrangement from C. violaceum or pRV2 (Silva-Rocha and de Lorenzo, 2011) empty plasmid. To measure promoter activity, freshly plated single colonies were grown overnight in LB media, washed, and resuspended in fresh M9 media. Ten microliters of culture was assayed in 96-well microplates in biological triplicate with 170 µL of M9 media supplemented with required antibiotics and different AsIII or benzoate concentrations. Cell growth and GFP fluorescence was quantified using a Victor X3 plate reader (PerkinElmer, Waltham, MA, USA) and calculated as arbitrary units by dividing the fluorescence level by the optical density at 600 nm (reported as GFP/OD600) after background correction. Background signal was evaluated with the same strain harboring the pMR1 (Guazzaroni and Silva-Rocha, 2014) or pRV2 (Silva-Rocha and de Lorenzo, 2011) empty plasmid. Unless otherwise indicated, measurements were taken at 30 min intervals over 8 h. All experiments were performed at least three times. Raw data were processed using ad hoc R script1 and plots were constructed using Microsoft Excel, R, or MeV2.

### Table 1: Strains, plasmids, and primers used in this study.

| Strains, plasmids, and primers | Description | Reference |
|--------------------------------|-------------|-----------|
| Strains                        |             |           |
| Chromobacterium violaceum ATCC12472 | Wild type strain of C. violaceum | ATCC collection |
| E. coli DH5α                   | F′ lacZAM15A (lacZYA-argF)U169 recA1 endA1 hsdR17 R− M− supE4 thi gyrA relA | Sambrook et al., 1989 |
| E. coli W3110                 | K12 F− (mD-mE), ars mutant strain | Carlin et al., 1995 |
| E. coli AW3110                | K12 F− (ars:cam IN (mD-mE), ars mutant strain | Bachmann, 1972 |
| Plasmids                      |             |           |
| pMR1                          | CmR: oriR15a; promoter probe variant of pRV2 | Guazzaroni and Silva-Rocha, 2014 |
| pMR1-Parcvi::arsRcvi          | CmR: pMR1 inserted with the operator, promoter and regulatory region as native arrangement from C. violaceum | This study |
| pMR1-Parscvi::arsRcvi         | CmR: pMR1 inserted with the operator, promoter and regulatory region as native arrangement from E. coli | This study |
| pRV2                          | KmR, oriR15a; dual promoter probe vector with GFP and mCherry reporters | Silva-Rocha and de Lorenzo, 2011 |
| pRV2-Parscvi                  | KmR, Parcvi-GFP transcriptional fusion | This study |
| pRV2-Parscvi                  | KmR, Parcvi-GFP transcriptional fusion | This study |
| pSEVA438                      | SmR, ori pBAD1; expression vector based on the benzoate inducible xylS-Pm system | Silva-Rocha et al., 2013b |
| pSEVA438-arsRcvi              | SmR, pSEVA438 inserted with arsRcvi gene | This study |
| pSEVA438-arsRcvi              | SmR, pSEVA438 inserted with arsRcvi gene | This study |
| Primers*                      |             |           |
| 5arsecEco                     | GCGCGAATTCGCCCAAGTGAAGAAATCCG | This study |
| 3arsecBam                     | GCGCGAATTCGCCAATACATATGGCTTCCC | This study |
| 5arscviEco                    | GCGTGAAATTCGAGTTGCGGC | This study |
| 3arscviBam                    | GCGGAATTCCTGGCAGATGGTGAAG | This study |
| 5arsRecvi                     | GCGGAATTCGAGAAATGAAATGAAATGGTGAAGTGT | This study |
| 3arsRecBam                    | GCGGAATTCGAGAAATGAAATGAAATGGTGAAGTGT | This study |
| 5arsRecEco                    | GCGGAATTCGAGAAATGAAATGAAATGGTGAAGTGT | This study |
| 3arsRecBam                    | GCGGAATTCGAGAAATGAAATGAAATGGTGAAGTGT | This study |
| 5arsecBam                     | GCGGAATTCGAGAAATGAAATGAAATGGTGAAGTGT | This study |

*Restriction sites are underlined in the primer sequence.
FIGURE 1 | Analysis of the natural ars circuits from *Escherichia coli* and *Chromobacterium violaceum*. (A) Schematic representation of the feedback circuit controlling GFP/via expression. (B) Induction of the ars system from *E. coli* and *C. violaceum* on agar plates using increasing concentrations of As^{III} for 6 h. The left panel represents bacterial colonies grown in agar plates and at the right panel the GFP intensities were converted to a false color scale to facilitate the visualization of the differences. (C,D) Induction kinetics of the ars system from *E. coli* (C) and *C. violaceum* (D) in wild type (W3110) *E. coli* in M9 liquid media exposed to increasing concentrations of As^{III}. Solid lines represent the average of three independent experiments, whereas dashed lines represents the upper and lower limits of standard deviations.

**RESULTS**

The Natural ars System from *C. violaceum* Displays Enhanced Induction by As^{III}

In order to characterize the arsenic response system, we initially focused on the response system from *C. violaceum*, an environmental bacterium with low tolerance to arsenic (Azevedo et al., 2008; Silva-Rocha et al., 2013a). Because this bacterium is sensitive to micromolar doses of As^{III}, we reasoned that in the natural environment, this organism would have to trigger a strong transcriptional response to very low concentrations of this metalloid and thus, would be endowed with an intrinsically sensitive ars response system. In order to compare the performance of the ars system from *C. violaceum* with that of *E. coli* (the prototype system used for arsenic biosensor construction), we cloned the Pars/arsR elements from both organisms into a GFP reporter vector and introduced the resulting construct into *E. coli* in M9 liquid media exposed to increasing concentrations of As^{III}. This allowed us to faithfully assess the response of the native systems (i.e., retaining the negative feedback loop, Figure 1A) in the same bacterial host.

As seen in Figure 1B, the Pars/arsR system from *C. violaceum* had a higher GFP output upon exposure to 75 µM of As^{III} when assayed on agar plates compared to the *E. coli* system. In order to analyze the two systems quantitatively, we performed induction experiments in liquid minimal media and quantified GFP production at fixed time intervals. When we compared the
performance of the two systems, we observed that the Pars/arsR genes from *C. violaceum* displayed GFP induction dynamics significantly higher than that from *E. coli* (Figures 1C,D). It is worth mentioning that the concentration of 125 μM produces a reduced output due to the strong toxicity of arsenite to the strain. This result confirms our hypothesis that the Pars/arsR system from *C. violaceum* has a more efficient transcriptional response to AsIII. Taking into account the architecture of the *ars* regulatory elements, the results observed in Figure 1 could be due to differences in three parameters of the two systems. First, the Parscvi promoter could have a stronger intrinsic activity than Parseco. In this scenario, releasing ArsR repression would allow increased promoter activity at Parscvi. Second, ArsRcvi could have stronger binding affinity by its target DNA sequence than ArsReco. If that were the case, the feedback loop would stabilize in lower amounts of ArsR in *C. violaceum*, which could be easily inactivated by changes in AsIII concentrations, leading to higher promoter output. Finally, ArsRcvi could have higher affinity for AsIII and thus, small changes in concentration of the inducer would lead to increased inactivation of the repressor, resulting in higher promoter activity. In order to investigate these possibilities, we conducted a number of in vivo experiments to quantify the relative promoter strengths, the apparent repressor-promoter binding affinities, and the repressor-effector interactions as described in next sections.

**Parscvi and Parseco Have Similar Strengths with Different Kinetics in *E. coli***

In order to understand the molecular differences leading to the observed behavior of the *ars* systems from *C. violaceum* and *E. coli*, we first compared the relative promoter strengths in the absence of repression. For this experiment, Parscvi and Parseco were cloned upstream of a GFP/lva reporter gene and introduced into wild type and *ars* mutant strains of *E. coli*. As shown in Figure 2A, both promoters displayed similar maximal activities in wild type and mutant *E. coli*, indicating that promoter activity alone could not explain the differences observed in Figure 1. When we compared promoter dynamics alongside the growth curve, we observed that Parscvi displays high initial activity that tends to stabilize after 2 h of growth, whereas Parscvi activity increases dramatically in the first 4 h and then reaches a similar steady-state level (Figure 2B).

**The ArsRcvi Regulator Has a Higher Apparent Affinity for Its Target Promoter**

Once we determined the maximal transcriptional activities of both systems, we investigated the effect of increasing concentrations of ArsR on the activity of Parscvi and Parsesco promoters. We constructed an uncoupled inducible system (Figure 3A) where ArsR production is under the control of a benzoate inducible system based on the XylS/Pm element (Blatny et al., 1997). The specific Pars promoter is fused to a GFP reporter gene in order to provide a fluorescence output. In this system, the RBS sequences for both regulators are changed to the *tir* element, thus ensuring similar translation rates for both proteins. Using this setup, we induced the cells with increasing concentrations of benzoate to generate a stepwise decrease in promoter outputs (Figures 3B,C) (Brewster et al., 2014). Assuming that both ArsReco and ArsRcvi are produced from the same transcription and translation signals, the protein level produced should be equivalent and thus, should allow us to indirectly infer the

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**FIGURE 2 | Quantification of the intrinsic Parscvi and Parsesco activities.** Wild type (wt) *E. coli* or mutant (Δars) strains harboring the reporter plasmids containing the GFP/lva gene under the control of the specific promoters were assayed in M9 media in the absence of AsIII. Empty pRV2 plasmid was used as control. (A) Maximal activity of the specific promoter after 6 h of growth. (B) Promoter dynamics during growth of *E. coli AW3110* (Δars) harboring pRV2-Parsecvi (Cvi, blue) or pRV2-Parseco (Eco, red). Solid lines represent the average of three independent experiments, whereas dashed lines represents the upper and lower limits of standard deviations.
**FIGURE 3** | *In vivo* titration experiments using a benzoate inducible system. (A) Schematic representation of the circuit used. In this system, the target regulator is placed under the control of the XylS/Pm expression cassette from pSEVA438 (Silva-Rocha et al., 2013b). To ensure similar levels of ArsR regulators were produced, a strong RBS element was introduced between the Pm promoter and the start codon of the gene. A second plasmid based on pRV2 was used where expression of a GFPvlva reporter gene is placed under the control of the target promoter. Promoter activities were assayed during the growth curve analysis using increasing concentrations of benzoate (Bz, from 0 to 250 µM). (B) *In vivo* titration of ArsR/Pars from *C. violaceum*. (C) *In vivo* titration of ArsR/Pars from *E. coli*. Gray lines represent control strains harboring the empty pSEVA438 vector (i.e., no ArsR production). (D,E) Repression kinetics of Pars\(_\text{cvl}\) (blue) and Pars\(_\text{eco}\) (red) upon exposure to increasing benzoate concentrations after four (D) or six (E) hours of induction. Solid lines represent the average of three independent experiments, whereas dashed lines represents the upper and lower limits of standard deviations. Statistics differences are highlighted by (*) as analyzed using Student’s t-test with p-value \( p < 0.05 \).
binding affinity of each repressor for its target DNA sequence, similarly to the approach used by Wang and coworkers (Wang et al., 2015). The results are shown in Figures 3D,E, which represents the effect of increasing benzoate concentrations on target promoter activity at 4 and 6 h, respectively. These in vivo titration experiments indicate that Pars_{Cvi} has a higher decay in activity with increasing benzoate concentration. These results show that ArsR_{Cvi} has a higher in vivo affinity for its target DNA. By comparing the benzoate concentration required to reduce promoter activity in each system, we find that approximately five times more inducer is required to reduce Pars_{Eco} activity than Pars_{Cvi} activity (62 μM vs. 12 μM).

**The ars System from C. violaceum Shows Stronger Transcriptional Response to Arsenic**

Once we observed that the ArsR repressor from C. violaceum has a higher apparent affinity for its target than the ArsR repressor from E. coli, we analyzed the effect of arsenic binding during allosteric derepression of the system. We used the maximal benzoate concentration (250 μM) to ensure the ArsR concentration was high enough to fully repress Pars_{Cvi} and Pars_{Eco}. Using this setup, we exposed the reporter strain harboring the circuits from Figure 3A to increasing concentrations of As\textsuperscript{III}. As shown in Figure 4A, under saturating regulator concentrations, the ars system from E. coli is gradually induced with As\textsuperscript{III} concentrations above 0.25 μM. However, under similar conditions, the ars system from C. violaceum is insensitive to As\textsuperscript{III} concentrations below 1.0 μM (Figure 4B). This differential induction behavior could be observed in the dose-response curve for both systems at 6 h post-induction (Figure 4C) where the ars system from C. violaceum is insensitive to As\textsuperscript{III} concentrations below 1.0 μM and displays pronounced induction above this threshold. This remarkable difference between the responses of the systems indicates that the ars from C. violaceum displays steeper induction kinetics, whereas the system is completely OFF at low concentrations of inducer but displays strong induction upon reaching a certain threshold. In order to test whether this behavior was the result of the high level of ArsR_{Cvi} produced (due to the elevated benzoate concentration used), we performed induction experiments in which we varied both benzoate and As\textsuperscript{III} concentrations. As shown in Figure 5, the ars system from E. coli displayed a gradual induction by As\textsuperscript{III} under varying benzoate concentrations, whereas the induction of the C. violaceum system presented steeper slope even at reduced benzoate concentrations (and thus low ArsR_{Cvi} levels). Taken together, these results indicate that the differences in the induction profiles of both systems are the result of intrinsic differences in the way the ArsR regulators interact with As\textsuperscript{III}.

**DISCUSSION**

Bacteria able to thrive in contaminated environments are endowed with very efficient detoxification mechanisms controlled at the gene expression level. In this context, different metalloregulatory proteins have evolved to control
gene expression in response to different metals and metalloids. In vivo functional characterization of the ars system from several bacteria has revealed strong variation in the number of resistance genes, operon organization and, final resistance levels (Carlin et al., 1995; Paez-Espino et al., 2009). However, a conserved feature of the ars system is that the first gene of the operon encodes the ArsR regulator, which repress its own expression in a feedback loop (Wu and Rosen, 1993; Xu and Rosen, 1997). The ArsR from both E. coli and C. violaceum are members of the SmtB/ArsR family. This class of transcription factors include small proteins that share a core secondary structure formed by five alpha helixes and two anti-parallel beta strands in the form ααααββα (Cook et al., 1998; Busenlehner et al., 2003). Although DNA binding occurs through the helix-turn-helix domain formed by helix three and four, members of this family have a more degenerate inducer-binding site that could be located in helix three (Type 1 regulators – ArsReco) or in helix five at the dimerization interface of the protein [Type 2 regulators – ArsRcvi (Qin et al., 2007)]. Considering the metal binding site, ArsR proteins have a conserved metalloid-protein interaction interface, in which cysteine residues are required to coordinate the ligand (Busenlehner et al., 2003). ArsReco has an AsIII binding site formed by Cys residues located at helix three (Wu and Rosen, 1993), whereas ArsRcvi belongs to the class of regulators where binding is formed by Cys residues at helix five (Qin et al., 2007; Azevedo et al., 2008). These differences between the two classes of regulators also imposes differences on the intramolecular allosteric switch upon inducer binding because type 1 regulators have the ligand-binding site close to the DNA binding domain, whereas signal transmission in type 2 regulators must occur from a distance (Qin et al., 2007).

While differences in the localization of metalloid binding site of the regulatory proteins might account for the different expression behaviors observed, other parameters such as protein-DNA interaction might play an important role in the process. For instance, Li et al. (2015) have used in vitro mutagenesis to tune the arsenic response of the ars system from the R773 plasmid of E. coli. By analyzing the variants with enhanced performance, they found several mutations into the ArsR binding site (ABS) at the DNA. This found is particularly relevant since, while members of the SmtB/ArsR family recognize palindromic DNA sequences, the ABS sequence found in the ars promoter of E. coli has an imperfect palindromic sequence (Wu and Rosen, 1993). As the ars systems from E. coli and C. violaceum have no conserved ABS sequence (Carepo et al., 2004; Azevedo et al., 2008), this difference on protein-DNA interface may explain the stronger binding affinity of ArsRcvi and the difference in the dynamics of gene expression. Altogether, these structural differences between the two regulators analyzed here could explain the discrepant behavior (i.e., gradient vs. steeper) during expression of the ars systems.

While the ArsR from E. coli proved to be more sensible to lower arsenic concentrations when assayed in the decoupled system (Figures 4 and 5), the C. violaceum system displayed a better triggering system at higher concentrations, with a steeper slope promoter induction. Although the particular features of the two classes of regulators have not been systematically investigated, different molecular mechanisms for an allosteric switch induced by AsIII binding could be the reason for the different induction profiles observed for ArsR regulators of C. violaceum and E. coli. Uncovering these mechanisms should be a target of future research in order to further understand...
the evolution of SmtB/ArsR protein family members and for biosensor development.

Microorganisms are a valuable source of molecular components for the construction of biological circuits for biotechnological applications (Cheng and Lu, 2012; Brophy and Voigt, 2014). From a historical perspective, most synthetic circuits constructed in bacteria have been implemented using components from the model organism E. coli (Church et al., 2014). This has indeed been the case for biosensors designed to detect arsenic in environmental samples with several designs having been constructed by shuffling the $ars$ components of E. coli (Siegfried et al., 2012, 2015; Wang et al., 2015; Merulla and van der Meer, 2016). Since the World Health Organization (WHO) recommended acceptable limit for drinking water of 10 $\mu$g/L arsenic, any modification of natural arsenic sensing systems for biosensing purposes should aim increasing responsivity of the systems to concentrations close to this limit. The characterization of the $ars$ components of an environmentally relevant bacterium, C. violaceum, shows that orthologous systems dedicated to the $ars$ biosensing purposes should aim increasing responsivity of the system in bacteria with such an atypical induction profile, which this is the first report of a naturally occurring arsenic response profile makes these components very attractive for novel designs of arsenic biosensing. Additionally, to the best of our knowledge, this is the first report of a naturally occurring arsenic response system in bacteria with such an atypical induction profile, which provides new venues for the investigation of $ars$ evolution and for the application of this pathway in biosensor design.

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AUTHOR CONTRIBUTIONS

RS-R and LA designed the experimental strategy. LA and LM performed the experiments. LA, LM, and RS-R analyzed and interpreted the data. LA and RS-R wrote the manuscript. All authors have read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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