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Genome Sequence of *Brevundimonas* sp., an Arsenic Resistant Soil Bacterium

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Abstract: *Brevundimonas* sp. is a bacteria able to grow in metal(loid) contaminated soil from Puchuncaví Valley, central Chile. This study has isolated a bacterial strain capable of growth under high doses of arsenic (As) (6000 mg L^{-1}), and a draft genome sequence was generated. Additionally, real-time PCR was performed to examine the effect of As on some genes related to As resistance. Results demonstrated a total of 3275 predicted annotated genes with several genes related to the *ars* operon, metal(loid) resistance-related genes, metal efflux pumps, and detoxifying enzymes. Real-time PCR showed that the *arsB* involved in the efflux of As was down-regulated, whereas *arsR*, *arsH*, and *ACR3* did not show differences with the addition of As. Our study provides novel evidence of diverse As regulating systems in tolerant bacteria that will lead to a better understanding of how microorganisms overcome toxic elements and colonize As contaminated soils and to the possible use of their specific properties in bioremediation.

Keywords: *Brevundimonas* sp.; arsenic; draft genome; metal(oid)s tolerance

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1. Introduction

Arsenic (As) is a metalloid ubiquitous in the environment with origins from natural sources and anthropogenic activities [1]. These activities include the burning of fossil fuels and the use of agricultural pesticides and fertilizers, which contribute to the abundance of As in the environment [2]. The As metalloid is potentially harmful to human health in cases of direct contact and bioaccumulation, causing several diseases and different pathologies ranging from neurological disorders to the development of cancer [3–5]. In ecosystems, As can affect plant development, growth, and productivity due to an abundance of morphological, physiological, biochemical, and molecular alternations [6], such as reduced root elongation, suppression in the number of leaves and leaf area, thereby reducing photosynthesis and biomass accumulation [7], in addition, reduces the rate of photosynthesis and interferes with the synthesis of chlorophyll [8]. Also, inactivate key enzymes through interaction with sulfhydryl groups (-SH) or by replacement of ions from their active sites [9]. Furthermore, it has deleterious effects on carbohydrate and lipid metabolism, among others [6].

The toxicity of As to plants depend on the speciation of the metalloid in the environment. Such toxicity will depend on factors such as redox potential, pH, organic matter, and moisture content, all of this determining its mobility in the soil [10]. As can exist in

both organic and inorganic forms. The inorganic forms arsenate (AsV; AsO_4^{3-}/H_3AsO_4) and arsenite (AsIII; AsO_3^{3-}/ H_3AsO_3) are more toxic than organic forms like arsenobetaine or arsenosugars that are recognized as non-toxic [11,12]. The most dangerous biochemical effect of As at the subcellular level is the production of the reactive oxygen species (ROS) such as superoxide radical (O_2^-), hydroxyl radical (OH), and hydrogen peroxide (H_2O_2) [13,14], and can cause unrepairable damage to important macromolecules, including lipids, proteins, carbohydrate, and DNA [15,16].

Microorganisms have developed different mechanisms such as methylation, reduction, and oxidation to transform AsIII to the less toxic form AsV [17]. Microbial-mediated processes involved in the reduction or oxidation of As are environmentally important since they can produce a change in the oxidation state, thereby changing the bioavailability and toxicity [18,19]. Several authors [20–22] reported that microbes that can withstand a high concentration of As could be potentially used for the bioremediation of As.

For As-resistant bacteria, the As detoxification processes mainly depend on their *ars* operons [23]. Resistance mechanisms to As involve an influx/efflux system that is encoded by the *ars* operon. The two most common types of these operons contain either five (*arsRDABC*) or three (*arsRBC*) genes [24]. *ArsC* encodes an AsV reductase enzyme that reduces AsV to AsIII prior to efflux. The AsIII produced is exported out of the cell by an AsIII efflux pump encoded by *arsB*, often associated with an ATPase subunit, *arsA* [25]. *ArsR* and *arsD* have a regulatory role, with *arsR* encoding a repressor involved in the basal regulation of the *ars* operon, while *arsD* is a second repressor controlling the upper levels of *ars* genes expression [26].

Bacteria of the genus *Brevundimonas* have been isolated from a wide variety of environments and often exhibit the ability to promote plant growth [27]. Additionally, they may protect plants from heavy metals toxicity and other environmental stresses [28,29]. This study evaluated AsV resistance in a newly isolated *Brevundimonas* strain and identified the As and heavy metal-resistance genes based on draft genome sequencing and gene annotations.

The objective of this study was (i) to understand *Brevundimonas* As-resistant mechanisms and (ii) to explore its potential roles in As transformation in the environment.

2. Materials and Methods

2.1. Soil Sampling and Bacteria Isolation

The soil used for bacterial isolation was collected from the rhizosphere of three *Oenothera picensis* plants, located in the Puchuncaví Valley in the coastal area of central Chile (Valparaiso Region), 1.5 km southeast of the Ventanas copper smelter (32°46′30″ S; 71°28′17″ W). This plant was selected for being one of the few that grow in this place. This ecosystem has been subjected to large quantities of gaseous and metal-rich particulate pollution from the copper smelter since 1964 [30]. Among the metal(loid)s found in the soil are copper (Cu), zinc (Zn), lead (Pb) and As (385, 183, 135, and 52 mg kg^{-1}, respectively) [31].

To isolate the bacteria, 1 g of rhizospheric soil was agitated in sterile distilled water and shaken for 30 s; then serial dilution method was performed on Luria-Bertani (LB) agar plates (10 g tryptone, 10 g NaCl, 5 g yeast extract, 12 g agar per liter) supplemented with 50 mg L^{-1} of AsV ($Na_2AsO_4 \cdot 7H_2O$, Sigma–Aldrich, St. Luis, MO, USA). After 3–4 days of incubation, distinct morphotypes of bacteria were screened based on colony color, shape, and size. Each morphotype was purified by re-streaking on a fresh LB agar plate and the purified isolates were maintained at 4 °C.

2.2. Arsenic Resistance

To test As resistance, the strains were incubated in LB medium plus As(V), the concentrations used ranged from 500 to 8000 mg L^{-1}, in 500 mg L^{-1} increments, at 28 °C, 200 rpm. After 48 h of incubation, the OD600 was recorded. *Brevundimonas* sp. B10 was selected, subsequently identified, and used for the following assays.

2.3. Molecular Identification of Bacteria

The DNA extraction was performed by selecting the strain and incubating it in LB broth medium at 28 °C, 200 rpm for 48 h, and centrifuged at 8000× *g* for 7 min. The pellet was used for DNA extraction using the UltraClean® Microbial DNA Isolation Kit (MO-BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The 16S rRNA gene was amplified using PCR with universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGTTACCTTGTTACGACTT-3′) according to Banerjee, et al. [32] and sequenced in Macrogen (Seoul, South Korea). The resulting sequences were queried against the GenBank 16 rRNA database (http://www.ncbi.nlm.nih.gov/, accessed on 18 January 2018) using MOLE-BLAST to find the closest known sequences. The genome was also queried in automated multilocus species tree (autoMLST) [33] server to identify close genomes from NCBI database. The phylogenetic analyses of the *arsR*, *arsB*, and *arsC* genes of *Brevundimonas* sp. B10 and other bacteria containing the *ars* operon were performed by aligning the concatenated protein sequences of *arsR*, *arsB*, and *arsC* genes with the MUSCLE algorithm. The resulting alignment was used to find the best-fit substitution model by Maximum Likelihood, and gaps/missing data were treated with partial deletion with site coverage cut-off of 95%. The final tree was constructed with WAG + G model by Maximum Likelihood over 526 informative positions. The stability of the tree was evaluated by the bootstrap method with 1000 replications. All these analyses were performed in the Molecular Evolutionary Genetics Analysis software [34].

2.4. Growth Conditions and Genomic DNA Preparation for Sequencing

Brevundimonas sp. B10 was cultured in 10 mL of liquid LB medium at 28 °C for 48 h with 200 rpm shaking. For genomic DNA isolation, bacterial cells of 0.5 mL were collected by centrifugation at 20,000× *g* for 3 min. After removing the supernatant, the cell pellet was resuspended in 0.75 mL Sox1 solution and then transferred into a bead tube of Sox DNA Isolation Kit (Metagenom Bio, Waterloo, ON, Canada). Cell lysis was performed at 4 M/s for 40 s using the FastPrep-24 Instrument (MP Biochemicals, Santa Ana, CA, USA). Lysate was recovered by centrifuging at 20,000× *g* for 5 min and transferred into a 1.5 mL tube containing 5 µL of RNase A solution (10 mg/mL). Following incubation for 10 min, 1.5 mL of Sox solution 3 was added and mixed. Purification of genomic DNA using a spin column was carried out according to the supplier's instructions.

Genomic DNA concentration was quantified using a NanoDrop 2000 spectrophotometer (ThermoFisher). The quality of DNA prep was also checked with 0.8% TAE agarose gel.

2.5. Genome Sequencing and Assembly

The genome of *Brevundimonas* sp. isolate B10 was sequenced with the MiSeq platform (Metagenom Bio Inc., Waterloo, ON, Canada) using the Nextera DNA Flex Library Preparation Kit and the MiSeq Reagent Kit v2 in the paired-end mode for 251 cycles. Sequencing and quality control resulted in 942,471 high-quality reads used to create a de novo assembly of *Brevundimonas* sp. B10 strain using SPAdes v3.11 [35]. This assembly was used as the primary input to GFinisher [36] along with alternate de novo assemblies from MEGAHIT v1.1.2 [37] and ABySS v2.0.2 [38]. The resulting scaffolds were submitted to Rapid Annotations using Subsystems Technology (RAST) server (http://rast.nmpdr.org, accessed on 18 January 2018) [39] for their annotation where Prodigal [40] is used for gene calling. The completeness of the genome was assessed using BUSCO [41], with alphaproteobacteria_odb10 database as a query.

2.6. As-Resistance Related Genes Expression Assay

Brevundimonas sp. B10 was grown in LB broth amended with 0, 500, and 1000 mg L^{-1} AsV, and incubated at 28 °C under shaking conditions (200 rpm) for 48 h. Culture without the addition of metalloid served as control. Experiments were conducted in triplicate with 2 technical replicates each. Total RNA was isolated using SV Total RNA Isolation System (Promega,

WI, USA) following the manufacturer's instructions. Final elution was made with 50 µL of DNase-free water (DEPC), and concentration for each sample was quantified in MaestroNano® spectrophotometer (MaestroGen, Hsinchu City, Taiwan). To avoid sample contamination with genomic DNA and false positives in the RT-qPCR, RNA samples (10 mg) were treated with an RNase-free DNase I Set (E.Z.N.A, Omega Bio-Tek, Norcross, GA, USA). The integrity and quality were verified by denaturing gel electrophoresis. RNA was stored at −80 °C until its use.

Reverse transcription was performed with 1 µg RNA using AffinityScript QPCR cDNA Synthesis Kit (Agilent Technologies, Santa Clara, CA, USA). Primers for real-time PCR, listed in Table 1, were designed using AmplifX 1.7.0 [42] based on the draft genome sequence determined in this study. The *rpoB*, *gap*, and *rho* genes were chosen as candidates for reference genes. Real-time PCR reaction mixtures contained 10 µL PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, Foster City, CA, USA), 10 µmoles of each primer, 2 µL template (10× diluted cDNA from cultures), and nuclease-free sterile distilled water to a total volume of 20 µL. The relative expression quantification was carried out in a StepOnePlus Thermocycler (Applied Biosystems). Thermal conditions were 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s, 60 °C for 15 s and 72 °C for 15 s. All reactions were performed in triplicate. Primers for real-time PCR analyses were designed from the sequences of *Brevundimonas* sp. B10 genome. Each primer was tested in silico in an attempt to avoid discard dimer formation. Target genes (*arsB*, *arsR*, *ACR3*, and *arsH*) are related to As-resistance.

Table 1. Set of primers used in this study for the relative expression assay of genes involved in arsenic resistance in *Brevundimonas* sp. B10. * Housekeeping genes.

Gene	Sequence 5′-3′	Length (pb)
arsB	GGGCATGTATCTGGTGGTCT AGGACAGTCGGCATGTTGTT	165
arsR	CGGTGACATCTCCAACCATC TCCTTGAGCAGGAAGAGGAC	165
ACR3	ATCAGCCGGAATACATGACC CCAGGCATAAAGGCTGAAGA	164
arsH2	CTTCCAGGAGTTCGACGG CGGACCAGAATGGTGAAG	165
* *rpoB*	CATCTATCGCCTGTCGAAGTTCCA GTCTTCGAAGTTGTAGCCGTTCCA	196
* *gap*	GACCATCGTCTACAAGGTCAACCA GATCCTTGTGCATCGTATCCAG	196
* *rho*	AACATCGCCAAGTCGATCGAGA GCTTGGCCTTTTCGATCACCAT	187

2.7. Data Analyses

geNorm™ [43] and NormFinder [44] algorithms were used to evaluate the stability of candidate reference gene expression using the Ct values transformed to relative quantities as input. Gene expression data were compared by analysis of variance followed by Tukey's multiple range test. Statistical analyses were conducted using StatSoft Inc. (2004) STATISTICA (Data Analysis Software System), Version 7.

3. Results

3.1. Bacteria Identification and As-Resistance

We selected the strain *Brevundimonas* sp. B10 for the following experiments due to the high As-resistance and the demonstration in previous assays that it can increase tolerance to heavy metals in wheat plants [31]. The selected bacteria showed 100% identity with *Brevundimonas intermedia* (Accession number KR811205.1), meanwhile, the genome evaluated in autoMLST showed that the closest genomes in the NCBI database measured as Average Nucleotide Index (ANI) are from *Brevundimonas* genus, having *Brevundimonas*

sp. assembly code GCF_001422455 the highest ANI of 90.5%. The minimum inhibitory concentrations (MIC) of the strain was 6000 mg L^{-1} of AsV.

The phylogenetic tree (Figure 1) of *ars* genes of *Brevundimonas* sp. B10 showed greater closeness to *Corybacterium efficiens* and *Acidithiobacillus ferridurans*.

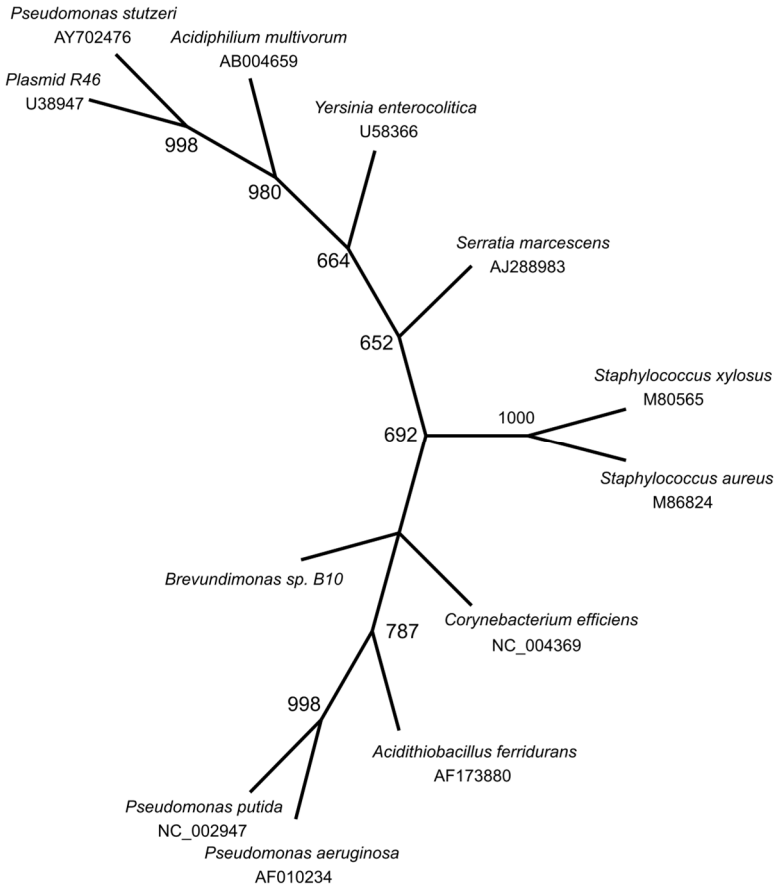

Figure 1. Phylogenetic tree of the *arsR*, *arsB*, and *arsC* genes of *Brevundimonas* sp. B10 and other bacterias containing the *ars* operon using WAG + G model by Maximum Likelihood over 526 informative positions. The stability of the tree was evaluated by the bootstrap method with 1000 replications.

3.2. Genome Properties

The draft genome sequence of *Brevundimonas* sp. B10 strain is 3,343,585 bp long contained in 7 scaffolds with a G + C content of 66.5% and an N50 value of 1,543,057 bp. The search for conserved orthologs genes (BUSCO) showed no missing BUSCO genes, and 99.5% completed BUSCO genes, indicating a high degree of completeness (Table S3). It has 3275 predicted genes including 3185 (97.25%) protein-coding genes, 58 (1.78%) RNA genes, and 32 (0.97%) pseudogenes. A total of 1391 (42.47%) genes were classified inside a SEED subsystem (Figure 2). More detailed information on the genome statistics is shown in Table S1. The genome sequencing project information is summarized in Table S2 and the sequences were deposited in the BioProject PRJNA505530 and the Genbank ID accession number is SPVH00000000.1.

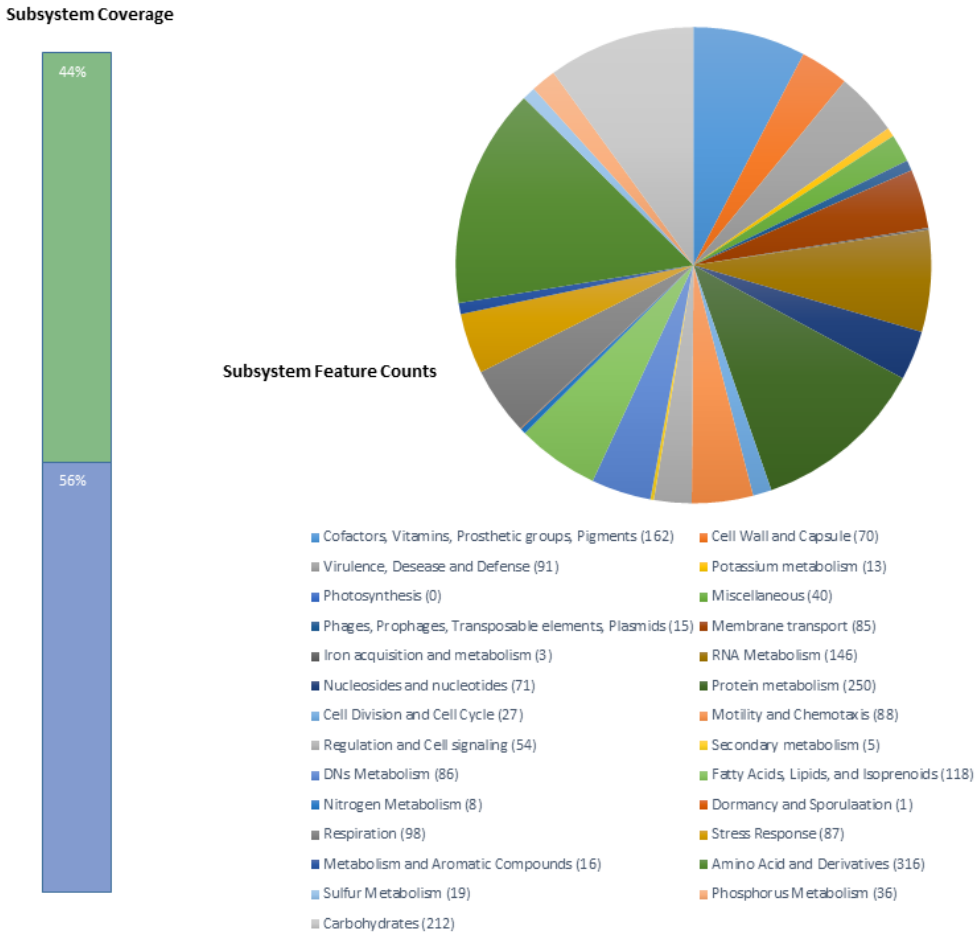

Figure 2. Subsystems distribution statistic of *Brevundimonas* sp. B10, based on RAST annotation server.

3.3. Insights from the Genome Sequence

Genome analysis showed 82 protein-coding genes associated with virulence, disease, and defense systems, among which, 62 belong to the defense subsystem and highlight those of resistance to metals that include Cu-Zn-Cd resistance, As resistance, Cu tolerance, resistance to chromium compounds, and multidrug resistance efflux pump (Table 2).

Table 2. Protein coding genes associated with the defense subsystem of *Brevundimonas* sp. B10.

Value	Defense Subsystems
7	Colicin V and Bacteriocin Production Cluster
21	Copper homeostasis
24	Copper-zinc-cadmium resistance
8	Arsenic resistance
2	Coper homeostasis: copper tolerance
4	Resistance to fluoroquinolones
5	Beta-lactamase
6	Multidrug-resistance Efflux pump
1	Resistance to chromium compounds

Among As-resistance genes (Table 3), we found two *ars* operons. One of the operons (*arsRBCH* type) is constituted by an arsenite-responsive transcriptional regulator gene (*arsR*), arsenite efflux pump gene (*arsB*), arsenate reductase gene (*arsC*), and NADPH-dependent flavin mononucleotide reductase gene (*arsH*) gene. The second operon (*arsRCH* (*ACR3*) type) contains an arsenite-responsive transcriptional regulator gene (*arsR*), an arsenate reductase gene (*arsC*), an NADPH-dependent flavin mononucleotide reductase

gene (*arsH*) gene, and one arsenite permease gene (*ACR3*). The genetic organization of the two arsenic-resistance operons is shown in Figure 3.

Table 3. List of arsenic resistance-related genes found in *Brevundimonas* sp. B10.

Putative Gene	Annotation	Size (aa)	Accession Number
arsH	Arsenic resistance protein	247	TFW12232.1
arsC	Arsenate reductase	142	TFW12233.1
arsR	Transcriptional regulator	112	TFW12234.1
arsB	Arsenic efflux pump protein	427	TFW12235.1
arsR2	Transcriptional regulator 2	111	TFW11216.1
arsC2	Arsenate reductase 2	141	TFW11215.1
ACR3	Arsenical resistance protein	361	TFW11214.1
arsH2	Arsenic resistance protein 2	250	TFW11212.1
arsC3	Arsenate reductase 3	88	TFW15101.1
arsH3	Arsenic resistance protein 3	252	TFW13512.1

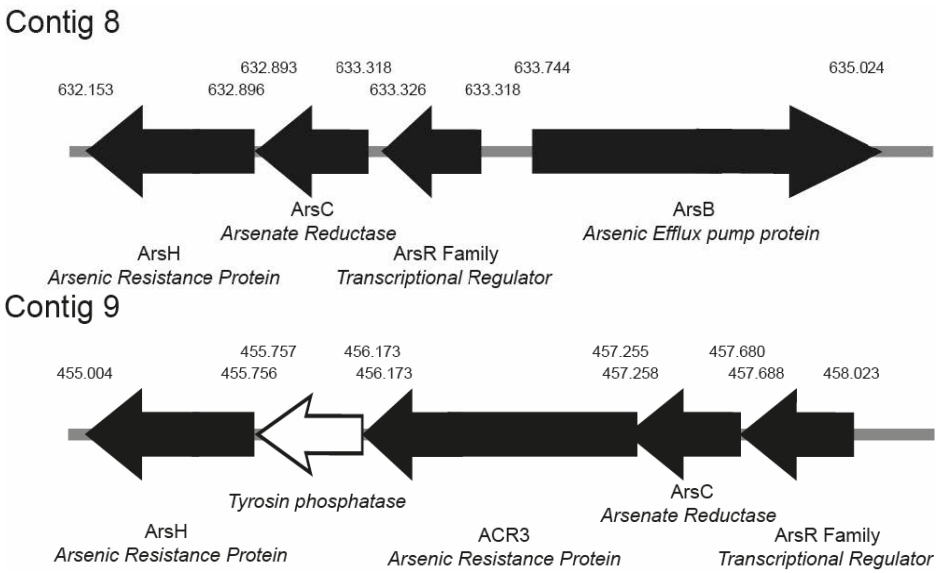

Figure 3. Genetic organization of the two arsenic resistance operon in strain *Brevundimonas* sp. B10. Gene orientations are shown by arrows.

Metal(loid)s resistance genes were also found in the annotation and are listed in Table 4. It was found that *Brevundimonas* sp. B10 had several genes related to heavy metal resistance, including Mg and Co efflux proteins, Co-Zn-Cd efflux transporters, and resistance proteins.

3.4. Real-Time PCR

The present study reveals the relative expression of *arsB*, *ACR3*, *arsH*, *arsC*, and *arsR* genes in *Brevundimonas* sp. B10 under As stress at different concentrations (500 and 1000 mg L^{-1}) analyzed by RT-qPCR (Figure 4). The relative expression of *arsH* was not induced significantly by the presence of As. The gene *ACR3* behave similarly to the control when was submitted to 500 and 1000 mg L^{-1} of As, the same happened to the relative expression of *arsR*. The relative expression of *arsB* was downregulated between 0.5 and 1 fold under the presence of As.

Table 4. List of heavy metal resistance-related genes found in *Brevundimonas* sp. B10.

Annotation	Size (aa)	Accession Number
Cobalt-zinc-cadmium resistance protein *CzcA*; Cation efflux system protein *CusA*	1062	TFW13601.1
Cobalt/zinc/cadmium efflux RND transporter, membrane fusion protein, *CzcB* family	420	TFW13602.1
Heavy metal RND efflux outer membrane protein, *CzcC* family	438	TFW13603.1
Lead, cadmium, zinc and mercury transporting ATPase (EC 3.6.3.3) (EC 3.6.3.5); Copper-translocating P-type ATPase (EC 3.6.3.4)	782	TFW13626.1
Copper resistance protein *B*	421	TFW13627.1
Copper resistance protein *CopC*	135	TFW13611.1
Copper resistance protein *D*	311	TFW13620.1
Copper resistance protein *CopC*	120	TFW13621.1
Heavy metal RND efflux outer membrane protein, *CzcC* family	452	TFW13623.1
Cobalt/zinc/cadmium efflux RND transporter, membrane fusion protein, *CzcB* family	433	TFW13624.1
Copper resistance protein *D*	311	TFW13486.1
Copper resistance protein *CopC*	120	TFW13487.1
Heavy metal RND efflux outer membrane protein, *CzcC* family	452	TFW13489.1
Cobalt/zinc/cadmium efflux RND transporter, membrane fusion protein, *CzcB* family	433	TFW13490.1
Cobalt-zinc-cadmium resistance protein *CzcA*; Cation efflux system protein *CusA*	1050	TFW13491.1
Lead, cadmium, zinc and mercury transporting ATPase (EC 3.6.3.3) (EC 3.6.3.5); Copper-translocating P-type ATPase (EC 3.6.3.4)	718	TFW13496.1
Cobalt-zinc-cadmium resistance protein *CzcA*; Cation efflux system protein *CusA*	1072	TFW13501.1
Cobalt/zinc/cadmium efflux RND transporter, membrane fusion protein, *CzcB* family	402	TFW13502.1
Heavy metal RND efflux outer membrane protein, *CzcC* family	417	TFW13503.1

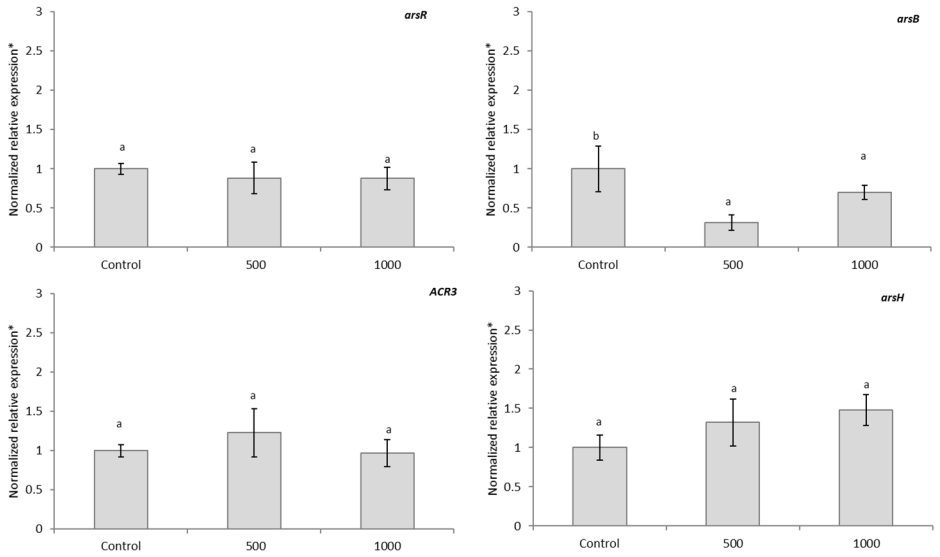

Figure 4. Relative expression of the *arsR, arsB, ACR3*, and *arsH* genes in *Brevundimonas* sp. B10 in LB broth spiked with 500 and 1000 mg L^{-1} of As. Standard deviation is indicated by different bars and letters show statistically significant differences ($p < 0.05$). * Normalized to *rho* and *rpoB*.

4. Discussion

The phylogenetic tree constructed with the genes belonging to the *ars* operon of *Brevundimonas* sp. B10 showed greater closeness to those of the genus *Corynebacterium*, *Acidithiobacillus*, and *Pseudomonas*, belonging to the phylum Proteobacteria and Actinobacteria. Further away are the genera *Staphylococcus*, *Serratia*, *Yersinia*, and *Acidiphilium*, all belonging to the phylum Proteobacteria, except for *Staphylococcus*, which belongs to Firmicutes. The distribution of arsenic resistance genes reflects the ubiquitous presence of

arsenic in nature, but they are present even in microbes isolated from putatively arsenic-free habitats [45].

The draft genome sequencing of *Brevundimonas* sp. B10 showed various defense subsystems related to heavy metals resistance, such as, As, Cu, Zn, and chromium (Cr), including the resistance proteins CopC, CzcC, CzcB, CzcA, among others. In previous experiments (data not shown), we observed that this strain, isolated from an environment contaminated with metal(loid)s, besides having high resistance to sodium arsenate (6000 mg L^{-1}), has moderate resistance to Cu (150 mg L^{-1}), Zn (200 mg L^{-1}) and Cr (150 mg L^{-1}). Growing evidence suggests that environmental organisms are a reservoir of resistance genes, including genes conferring resistance to antibiotics [46]. The high resistance to AsV might also be related to the widespread AsV resistance genes among bacteria and regardless of the level of As contamination, As-resistant bacteria appear to be phylogenetically diverse and widely distributed in the natural environment [47]. A considerable number of microorganisms are capable of resisting the toxic effects of As, using methods such as AsIII oxidation (to produce the less toxic AsV), extrusion of As from the cell, and minimizing the uptake of As from the environment [48]. The ability of *Brevundimonas* sp. B10 to resist a high concentration of AsV correlates with the observation in the draft genome of two operons, one with the *arsRBCH* arrangement and the other with *arsRCH* plus *ACR3*. Although the *arsRCBH* operon arrangement is not as common as *arsRBC* and *arsRDABC*, it has been widely detected in bacteria, such as *Salmonella enterica* [49], *Pseudomonas putida* [50], *Acidithiobacillus caldus* [51], *Acidothiobacillus ferrooxidans* [52], *Serratia marcescens* [53], among others. Thereby, the ars-resistance system seems to be a common bacterial mechanism for evading the toxic effects of AsIII and AsV. And more specifically referring to *Brevundimonas*, several As resistance genes are found in other species of this genus; for example, the *arsC* gene is found in *B. vesicularis* (accession number WP_066626785.1), *B. naejangsanensis* (WP_025977280.1), *B. nasdae* (WP_039243957.1), among others; the *arsB* gene is found in *B. vesicularis* (WP_066626995.1) and *B. nasdae* (WP_039244107.1); *arsR* is found in *B. nasdae* (WP_039247942.1), *B. vesicularis* (WP_066629834.1), among others.

To better understand how some of these genes behave under high concentrations of sodium arsenate, we studied the relative expression of *arsR*, *arsB*, *arsH*, and *ACR3* genes through real-time PCR. The relative expression of *arsR* did not suffer variations in the presence of AsV, this is likely to be associated with the fact that it is an inducible repressor that binds with the promoter region and regulates the *ars* operon in the presence of AsIII [54]. This means that the absence of AsIII represses *ars* transcription [55] and in the presence of AsIII, *arsR* binds to the effector and dissociates from the operator region allowing transcription to proceed [56].

Once AsV is taken up by the cell, it is reduced to AsIII and pumped to the external environment [24]. This enzymatic reduction of AsV can be performed by the proteins arsC, arrA, and ttr [57]. We did not detect arrA or ttr genes in this study, but we did detect three *arsC* genes. However, the in silico primer construction of the *arsC* gene was not successful, so the relative expression of *arsC* could not be tested. These data could have helped us understand our observed repression of the *arsB* gene. The reduced AsIII is transferred to *arsB*, which is often associated with *arsA*, an ATPase subunit, and forms an efficient transmembrane AsIII efflux pump [58]. In our study, the analysis of the draft genome of *Brevundimonas* sp. B10 did not identify an *arsA* homolog in the organism. Thus, the lack of *arsA* might reduce the efflux ability of *arsB*. Studies in *Campylobacter jejuni* showed that *arsB* contributes to AsIII resistance, but not AsV resistance [59]. Besides the extrusion mediated by *arsB*, bacteria also have other strategies for AsIII efflux like GlpF and Aqpz channels [45]. However, neither of the corresponding genes were found in the draft genome. Another AsIII transporter includes the gene encoding As efflux pump *ACR3* [60], but the presence of AsV did not affect the relative expression of this gene in *Brevundimonas* sp. B10. The *ACR3* pump may also couple with the *arsA* ATPase to form a more efficient primary AsIII efflux system [61].

Along with *arsM*, which was not found in the genome of *Brevundimonas* sp. B10, *arsH* expands the spectrum of microbial resistance of the *ars* operon from inorganic to organic arsenicals [45]. In this sense, the expression of *arsH* did not vary with the As amendment, this may be due to the absence of organic As or because the bacteria does not transform the inorganic As to organic.

5. Conclusions

Our results showed that two operons were found in the genome of *Brevundimonas* sp. B10, which encode *arsH, arsC, arsR, arsB*, and *ACR3* genes, which confer the ability to tolerate up to 6000 mg L^{-1} AsV. We also found several genes related to heavy metal tolerance, such as Cu, Zn, Cd, Pb, and others. This study provides insight into how this bacterium can survive in multi-contaminated soil with metal(oid)s and the potential use in bioremediation strategies.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/d13080344/s1, Table S1: Genome statistics, Table S2: Project information Genome statistics, Table S3: BUSCO results: The number of single-copy conserved orthologs found in the genome.

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