Isolation and Molecular identification of native As-resistant bacteria: As(III) and As(V) removal capacity and possible mechanism of detoxification

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Research Article

Keywords: Rhodococcus gordoniae, Microbacterium hydrocarbonoxydans, Exiguobacterium indicum, Pseudomonas kribbensis, Xichu, Ars C, Aio, Arr.

Posted Date: June 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-556252/v1

Version of Record: A version of this preprint was published at Archives of Microbiology on February 23rd, 2022. See the published version at https://doi.org/10.1007/s00203-022-02794-0.

Abstract

The study of arsenic resistant microorganisms with high arsenic removal capacity is fundamental for the development of economically sustainable technologies for the treatment of water contaminated with this metalloid. In this work, the isolation and identification of 4 native strains was carried out.: Rhodococcus gordoniae, Microbacterium hydrocarbonoxydans, Exiguobacterium indicum and Pseudomonas kribbensis . R.gordoniae was identified as the bacterium with the highest growth capacity in both As(III) and As(V). E.indicum removed about 74.8% of Arsenate, As(V), and 61.7% of Arsenite , As(III), while R.gordoniae removed about 81.6 % of As(III), and 77.2% of As(V), while that M.hydrocarbonoxydans was able to remove up to 79.9% of As(III) and 68.9% of As(V). Finally, it was observed that P. kribbensis removed about 80.2% of As(V). This study also contributes to the possible detoxification mechanisms employed by these bacteria, the knowledge of which could be crucial in the successful implementation of in situ bioremediation programs.

1. Introduction

Arsenic (As) is a toxic metalloid present in high concentrations in natural waters due to various hydrogeological, geochemical conditions and anthropogenic processes, causing serious environmental health problems worldwide (Kumari et al., 2019). One of the main problems is the use of As-contaminated water from groundwater or river water for drinking or domestic use (Arroyo-Herrera et al., 2021; Muñoz et al., 2016; Osuna-Martínez et al., 2020). The main critical effects associated with long-term ingestion of inorganic arsenic by humans are cancer, skin lesions, cardiovascular disease, neurotoxicity, renal diabetes, and hematological and respiratory disorders (Cavalca et al., 2013). Although the World Health Organization recommends 10 μgL^{-1} as the maximum concentration of As in drinking water as a permissible limit (World Health, 2018), several countries, including Mexico, India, and Bangladesh, continue to allow As from 25 to 50 μgL^{-1} (Kumari et al., 2019; Loredo-Portales et al., 2017). Around the world, it is estimated that more than 40 million people are at risk of drinking water contaminated with As (Nordstrom, 2002). In Europe, the Pannonian Basin (Hungary, Serbia, and Romania), more than 600,000 residents are at risk of drinking water containing high As concentrations. In Asia, countries such as India, Bangladesh, China, Pakistan, and Vietnam, evidence high concentrations of As contamination. In Africa, elevated As concentrations have been reported in both the surface and groundwater (Mudzielwana et al., 2020). In Latin America, the estimated population at risk of As exposure exceeds ~14 million people, with hundreds of recorded cases of exposure in countries such as Chile, Argentina, and Mexico (Castrejón et al., 2020; Muñoz et al., 2016; Osuna-Martínez et al., 2020). Within this context, in Mexico, it is estimated that approximately 450,000 people are regularly exposed to arsenic-contaminated water(Arroyo et al., 2013). For instance, in the Northeast of the State of Guanajuato high As levels have been detected in the municipality of the Xichu mining district (up to 62,302 μgg^{-1}), (Osuna-Martínez et al., 2020). The inorganic forms of As predominantly exist as trivalent arsenite ($As(OH)_3$, $As(OH)_4^-$, $AsO_2OH_2^-$ and AsO_3^{3-}) and pentavalent arsenate (AsO_4^{3-}, $HAsO_4^{2-}$, $H_2AsO_4^{-}$) in natural waters. Pentavalent species of As, As(V) are stable in oxygen rich aerobic environments, whereas the more soluble and toxic trivalent arsenite, (As(III)) is prevalent in moderately reducing anaerobic environments such as groundwater (Kumari et al., 2019; Prasad et al., 2011). As(III) is 70 times more toxic than methylated species and 10 times more toxic than As(V), which is poorly soluble in water and therefore less bioavailable. As(III) interacting with thiol groups of proteins and enzymes inhibiting their functions, is able to adhere to the surface of various minerals, such as ferrihydrite and alumina (Cavalca et al., 2013). Existing technologies for As removal are primarily based on physico-chemical methods such as precipitation and/or adsorption of As in the form of As(V),

lime softening, ion exchange, and membrane separation. Most of these methods involve the chemical preoxidation of As(III) to As(V), as the negatively charged arsenate adsorbs easily to solid surfaces, thereby facilitating precipitation and removal from contaminated matric. However, this may result in the production of undesirable by-products in such a way as to generate environmentally unfriendly technologies, in addition to the fact that they may be costly to apply. Bioremediation technique, which involves the use of microbes to detoxify toxic heavy metals or metalloids, has been receiving increasing attention in recent times as a means of cleaning up a polluted environment. Biosorption of metal ions is an example of a wide variety of potential and actual applications of the bioremediation technique in polluted water treatments (Pandey & Bhatt, 2016b). The use of microorganisms for the biosorption of As ions from water is an extremely efficient process. Bioaccumulation can also be considered as a second part of the process of metal and/or metalloid sequestration by living biomass (Kumari et al., 2019). In a geographical context, native bacteria inhabit contaminated areas. These possess the innate ability to tolerate high concentrations of As by the process of extrusion after microbial oxidation, reduction and methylation, collectively termed as biotransformation processes. Microbial transformation of As(III) to As(V) is an environment-friendly and economically viable alternative to the aforementioned treatment methods (Anna Corsini et al., 2014; Kumari et al., 2019). On the other hand, in bacteria, the genes involved in As metabolism include the *ars* (arsenic resistance system), *aox* (arsenite oxidase) and *arr* (arsenate respiratory reduction) operons, which can be found in both plasmids and chromosomes. Tolerance or transformation mechanisms in some bacteria include a chromosomal or plasmid coded *ars* operon with three or five genes. The operon includes a regulatory gene (arsR), a coding gene for a transmembrane ejection pump specific for arsenite (arsB), and a coding gene for an arsenate reductase (arsC) where the reduction from As(V) to As(III) is carried out by the same gene (Kruger et al., 2013). The *arr* operon includes *arr*A and *arr*B genes, in dissimilatory As(V)-reducing bacteria these genes are sometimes flanked by *ars* genes, forming clusters of arsenic-metabolizing genes in their genomes (arsenic-metabolizing gene island) (Tsuchiya et al., 2019). The objective of the present work was to analyze the resistance, growth and removal capacity of As(III) and As(V) by four native strains. These species were isolated, molecularly identified, and their genes (arsenic oxidizers and reducers) were amplified to propose a possible mechanism of detoxification against As. It is worth mentioning that this work is the first to investigate the As removal capacity of two little studied strains *P. kribbensis* and *M. hydrocarbonoxydans*, as well as their possible detoxification mechanism properties.

2. Materials And Methods

2.1 Study area and sampling. Water samples were collected along the Xichu River, within the mining area "La Aurora" corresponding to the municipality of Xichu, Gto, Mexico. The location points were AJ (21°19´40.04 ´´N,100°01´57.18´´O), J(21°19´48.13´´N, 100°01´57.89´´O), DJ (21°19´57.81´´N, 100°01´48.87´´O) and EJ (21°19 52.88” N, 100°01´54.09´´O), Figure 1. For microbiological sampling sterilized Falcon tubes were used. Each one was introduced into the body of water until it was filled and sealed before being removed from the body of water. At the same time, water samples for the physicochemical characterization were collected. For water sampling, the NMX-AA-14-1980 standard was taken into consideration. The plastic bottles used were first treated with HNO_3 10% for 48 h and washed with bi-distilled water. All samples were stored and kept under refrigeration until arrival at the laboratory.

2.2 Analysis of the water samples

2.2.1 Physico-chemical characterization. Samples were analyzed *in situ* according to the parameters established in the NOM-127-SSA1-2017. The quantifications of Fe^{2+}, S^{2-}, SO^{4-}, NO_3^{-1}, K^{+}, Mg^{2+}, $Mn^{2+}$and Zn^{2+}were done by handheld colorimeter (HACH DR 900®, USA). Temperature (°C), C.E (μScm^{-1}) and dissolved oxygen (O.D), were quantified by water analysis potentiometer, (Thermo scientific®, USA). The determination of total As was carried out by atomic absorption spectrometry.

2.2.2 Determination of total As concentration. All samples were digested with concentrated HNO_3 (Nitric acid 70%, purified by redistillation, ≥99.999% trace metals basis, Sigma Aldrich, USA) following the USEPA 3015A method (Microwave Assisted Acid Digestion of Aqueous Samples, USEPA North American Environmental Protection Agency), (Element, 2007). A microwave digestion system Titan MPS 8 Position Microwave Sample Preparation System MPS™ (model N3130110, Perkin Elmer, USA) was used. Deionized water ($18.2MWcm^{-1}$, resistivity) was used for all dilutions, obtained in a purification system (Milli-Q® direct 8/16 system, France). The flasks and all the material used in the preparation of the solutions were carefully cleaned in 15% HNO_3 for 48 h, rinsed in deionized water and dried in laminar flow. Quality control for the determination of metals was carried out by analysis of the standard reference materials (SRM), LGC6027 (Trace Elements in water). The calibration curve was established using the standard solutions prepared in 1mol L^{-1} HNO_3 by dilution from stock solutions (As Standard for AAS, TraceCERT®, 1000 mg/L As in nitric acid, USA). A PerkinElmer PinAAcle 900T Atomic Absorption Spectrometer equipped with MHS-15 Mercury/Hydride System (HG-AAS) was used in this study. High-purity argon (99.999%) was used as carrier gas (at a flow rate of 300 mL/min) as well as shielding gas (at flow rate of 700 mL/min). A hollow cathode lamp operating at 18mA was used and a spectral bandwidth of 0.7nm was selected to isolate the 193.7 nm As line. Peak height was used for quantitation. The detection limit (LOD) was calculated from the standard deviations of the blank solution (mostly nitric acid 0.2% of HNO_3), using the criterion of three times the standard deviations and ten times the standard deviations, respectively. All analyses were performed in the Environmental Engineering Laboratory of Sustainable Innovation of the UG-Guanajuato Campus (clean room, class 1000).

2.3 Isolation of strains in selective media with As. Nutritional Agar (NA) (BIOXON®, Mexico) was prepared with arsenate from sodium arsenate, Na_3AsO_4, (Sigma Aldrich-Merck, USA) and arsenite from sodium arsenite, $NaAsO_2$, (Sigma Aldrich-Merck, USA).The media were adjusted to pH 8.5 by adding 0.1 M NaOH (Fisher Scientific, USA) since it was the value quantified between the water samples at the sampling points. A 100 µl of water sample was inoculated to NA plates containing 3.5 mM of As(III) and As(V) respectively; the spread plate method was used to obtain single colonies. The plates were incubated under aerobic conditions at 30°C (Aguilar et al., 2020; Kumari et al., 2019). For sterilization of the media and the As salts solution, an autoclave (LAB-MED® model LMGV1M, Mexico) was used. Bacterial colonies with different forms, color and margins were purified on solid medium containing As at the same concentration, at least 3 times and stored at -80°C in a solution of 30% glycerol. The strains were characterized according to morphological characteristics: shape, margin, chromogenesis, optical detail and surface with the help of an optical microscope (Primo Star Zeiss GmbH, Germany). 3dp.rocks/lithophane software was used for image enlargement and analysis. Gram staining was performed with a Gram staining set (Millipore Sigma; cat REF 77730, USA) according to standard procedures (Gerhardt et al., 1994). Subsequently, from the isolated strains, one strain from each site was selected for the following analyses.

2.4 Molecular identification of As resistant isolates. The extraction of the genomic DNA of the bacteria was carried out, based on the protocol described by Murray and Thompson (Murray & Thompson, 1980), where the technique of extraction with Cetyl Trimethyl Ammonium Bromide (CTAB)/NaCl was used. Once the extraction was finished, electrophoresis was performed in a 1% agarose gel, stained with ethidium bromide to check the quality and concentration of the DNAg. All the reagents used were from the commercial company Sigma Aldrich-Merck.

2.4.1 Amplification procedure. Several concentrations of nucleic acid extracts were suspended in 5µl portions of water. These DNA dilutions were added in a PCR mix containing: 2.5 µl of 10X PCR buffer, 2 µl of direct and reverse initiators, Midex D and Midex R respectively, (final concentration, 1 µM each), 0.5µl of deoxynucleoside triphosphates (dNTPs) (final concentration, 200 µM), 2.0 µl of $MgCl_2$ (final concentration 2.0mM), and 3.0 U of Taq polymerase (Invitrogene®). Water Milli-Q ® was added, giving a final volume of 25 µl (Saiki, 1990) .The mixture was placed in a thermal cycler (Multigene LabNet®), with the following PCR conditions: initial denaturation at 95 °C for 3 min, 30 cycles of 95 °C for 30s, 59 °C for 30s and 72 °C for 3 min, followed by a final extension at 72 °C for 5 min (45). The purification of the PCR products was carried out with GE Healthcare® kit: GFX PCR DNA and Gel Band Purification, in which protocols and supplier suggestions were followed. Then, the purified PCR products were sequenced at the National Laboratory of Agricultural, Medical and Environmental Biotechnology (IPICYT), Mexico. Finally, the sequences were compared to those in the National Biotechnology Information Center (NCBI) database (Sayers, Beck, et al., 2021), using the BLASTn algorithm (the basic local alignment search tool).

2.4.2 Phylogenetic analysis. To obtain the most similar sequences of validly named bacteria, the 16S rRNA gene sequences of strains were compared with the available 16S rRNA gene sequences via a BLASTN 2.10.1 search. This is a program for comparing nucleotide sequences of DNA and/or RNA (Schwartz et al., 2000). This application is available on the NCBI website. A phylogenetic tree schematically reflects the degree of genetic kinship between bacteria based on their 16S ribosomal genes in order to obtain the correct sequence of each one of the ribosomal genes analyzed. The evaluation of the electropherograms and the alignment of the direct chain with the reverse was carried out. Multiple alignments of the sequences of the strain with other bacterial species were carried out, using the CLUSTAL X program (Larkin et al., 2007). The 16S rDNA phylogenetic tree was constructed using the distance method Neighbor-Joining (Saitou & Nei, 1987), that relates bacterial sequences from references selected in GenBank® (Sayers, Cavanaugh, et al., 2021).

2.5 Minimum inhibitory concentration of the isolated. The As(III) and As(V) resistance of the selected isolates was evaluated using minimum inhibitory concentration tests under aerobic conditions . The minimum inhibitory concentration (MIC) is defined as the lowest concentration of both inorganic As species that completely inhibits growth. MIC for four bacterial cultures were determined in nutrient broth medium (NB) (BIOXON®, Mexico) containing different concentrations of As(III) and As(V) , which ranged from 1 to 20 mM (Kumari et al., 2019). The medium was inoculated with cell suspensions taken from fresh cultures in the log phase and incubated at 120 rpm in an incubation shaker (Thermo Scientific MaxQ 4000, USA), at 30°C for 96 h. Two controls were prepared, for each experiment with As(III) and with As(V): (i) Nutrient broth supplemented with only bacteria (without arsenite or arsenate) (ii) Nutrient broth with arsenite or arsenate, and without bacteria. Optical density of cell cultures was taken at 24 h intervals for 96 h for determining the bacterial growth responses at different concentrations of As(III) and As(V). Bacterial growth was measured by determining the optical density at 600 nm (OD_{600}) in a Uv–vis Spectrophotometer (DR 3900, HACH, USA). Each experimental set up was prepared in triplicate.

2.6 Effect of pH on the growth bacteria culture. Yeast Extract Mannitol (YEM) broth (Merck, Germany) was prepared. The pH was adjusted from 3.5 to 12 by adding 0.1 M HCl or NaOH (Fisher Scientific, USA), and measured with a pH meter (Thermo Scientific Orion 4-Star, USA). It was then autoclaved at 121 °C for 20 minutes. The isolated strains were inoculated onto the medium and cultured at 30 °C in 250 mL Erlenmeyer flasks shaken at 120 rpm. Bacterial growth was measured by determining OD_{600} after a 24 h growth period.

2.7 Gene expression analysis. The presence of the arr (respiratory arsenate reduction system) and aox (respiratory arsenite oxidation system) genes was detected through their amplification using the DNA of the isolated bacterial strains (Chang et al., 2010). PCR amplification was performed with primers: arrA-CVF1 and arrA-CVR1 (direct and reverse respectively) for arsenate reductase A. As an initial step these primers were tested and evaluated in silico for virtual amplification of arrA genes in clones and subsequently for amplification and sequencing of arrA genes from groundwater samples as the monitoring of the arrA gene can provide useful information about the variations of the arsenate-reducing microbial species. The samples were then amplified with the primers: aoxA and aoxB (direct and reverse respectively) for arsenite oxidase AB. The primer “a” (5´-AATGACACCTTCACGGCG-3´), annealing 48 bp upstream of the aoxA stop codon, and primer “b” (5´-AGCACTCGATCTTTTGCAG-3´), annealing 872 bp downstream of the aoxB start codon, The amplification program was set to the following conditions: 94°C (initial denaturation temperature) for 3 minutes (1 cycle).The temperature was then cycled to 94°C for 30 sec., to 60°C for 40 sec., and then to 72°C for 1 min. This process was repeated for an additional 30 cycles, followed by a final extension at 72 °C for 10 min. Finally, the amplification of the genes was verified in a 1% agarose gel stained with ethidium bromide (Mirza et al., 2017).

2.8 Comparison of the growth profile among the four bacteria under As(III) and As(V) stress. The assays for growth kinetics were designed using a factorial design “A*B” of two-factor 8x4 (time and strain), where the “A” factor has eight levels (0-168 hours) and the “B” factor has four levels (A1, S3, A4, A11) respectively. The above was intended to identify the main effects of strains and time on microbial growth in the presence of As(III) and As(V). Growth kinetics were planned in 30ml of YEM broth, with an aliquot of each culture being taken every 24 hours. Three experiments were carried out as follows: 1) Growth kinetics without As, 2) Growth kinetics in YEM with As(III) and, 3) Growth kinetics in YEM with As(V). The concentration of arsenic was defined according to the result obtained by the MIC. Incubation conditions in Falcon tubes were inoculated with fresh bacteria culture at 30°C at 120 rpm for 168 h. Absorbance was measured at 600 nm in a Uv–vis Spectrophotometer (DR 3900, Hach, USA).

2.8.1 Statistical analysis. Initially, the determination of Normality (Shapiro-wilk test), Homogeneity (Bartlett test) and independence (Durbin Watson test) of the data obtained was carried out (Kirchman et al., 1982). The experiments in this study were performed in triplicate (n=3). The values of the Growth kinetics are given as means+-S.D using Microsoft excel software, version 2010. Data obtained from the factorial design were analyzed by a two-way analysis of the variance test (ANOVA) to identify statistically significant differences in the growth of bacteria in the presence of As(III) and As(V). Tukey's multiple range test was performed to identify the strains with the highest potential for interaction with As(III) and As(V), as well as their optimal growth times. Significance of differences was defined as $P< 0.05$, with a 95% confidence interval, using Minitab software, version 19.

2.9. Removal of As(III) and As(V) by isolated strains. Isolated strains that showed the best growth in the presence of As were selected and cultured under aerobic conditions. The concentration of As used was in relation to the

result obtained by the MIC. Synthetic water was prepared from the analyzed constituents of the water samples from the studied site based on the average concentrations of the results obtained (section 2.2.1), All the reagents used were from Sigma Aldrich. Bacterial pellet was collected and lyophilized as described (Kao et al., 2013): Biosorption assays for As(III) and As(V) were performed using the lyophilized cell pellet of the selected strains. From each strain, the lyophilized cell pellet (5 g/l) was added to 125 ml Erlenmeyer flasks with 30 ml of culture medium containing As(III), pH was adjusted to 8.5 by adding dilute solutions of NaOH, while the contact time was 168 minutes. The flasks were shaken (120 rpm/min) at 30 °C. Samples were withdrawn every 24h and centrifuged at 4000 rpm at 4°C for 15 min. In parallel, the same procedure was performed for the case of As(V). The supernatants were analyzed to determine the concentrations of As(III) and As(V) by the methodology described in section 2.9.1. All experiments were conducted in triplicate. The removal of As(III) and As(V) in percentages was calculated by using Eq. (1), (Kumari et al., 2019).

$$\text{Bioremoval As(III) or As(V)} \ \% = \frac{Ci - Cf}{Ci} \times 100 \qquad (1)$$

where,

Ci - Initial concentration of As(III) or As(V)

Cf - Final concentration of As(III) or As(V)

2.9.1 Analytical method. The determination of As levels was performed by HG-AAS described in section 2.2. The methodology carried out for the determination of As(III) and As(V) was by the combination of coprecipitation and atomic absorption spectrometry by hydride generation, as described in a previous work (Tuzen et al., 2009). As(V) was quantitatively recovered on aluminum hydroxide precipitate. After the oxidation of As(III) by using dilute KMnO4 (Sigma Aldrich-Merck, USA), the developed coprecipitation was applied to determine total As. As(III) was calculated as the difference between the total As content and As(V) content. The limits of detection (LOD) were based on three times the blank sigma (n=20). The same certified reference materials were used for method validation as described in section 2.2.2.

2.10 Analysis of Possible mechanism of biological removal of Arsenic. A bioinformatic search of the genes of the isolated species was carried out from the databases of the National Center for Biotechnology Information (NCBI).This platform includes the database (BD) Public library called GenBank® , which contains an extensive collection of nucleotide sequences obtained from more than 300,000 species, (Sayers, Cavanaugh, et al., 2021).

3. Results And Discussion

3.1 Physicochemical characterization and determination of total As in water samples. The results of the water characterization are shown below in Table 1. The average pH value obtained in the water samples was 8.73 $\pm$0.52, which shows that within the sampling region in the area of the "La Aurora" mine, a highly alkaline medium predominates. The average values for the parameters of C.E, O.D and temperature were 725.25 $\pm$ 155.06 μScm^{-1}, 4.75$\pm$ 0.66 mgL^{-1} and 28.73 $\pm$ 0.96 °C, respectively. For K^+, Mg^{2+}, Mn^{2+}, Fe^{2+}, Zn, SO_4^{2-} , NO_3^- were 1.97 $\pm$ 0.67, 33.55 $\pm$3.17, 0.20 $\pm$ 0.16, 0.05 $\pm$ 0.02, 0.03$\pm$0.01, 136.88 $\pm$ 21.15 and 4.64 $\pm$ 4.27 mgL^{-1}, respectively. Table 2

shows the As values found in the water samples from the four sampling points (AJ, EJ, J, and DJ). The analytical parameters obtained by the HG-AAS method are shown at the bottom of the table. The mean As concentration was 58.48±33.68 μgL^{-1}, with the maximum value being 108±2.1 μgL^{-1} (DJ) and the minimum 35±0.2 μgL^{-1} (AJ). This heterogeneity in element concentration in different samples is common in mining areas due to constant movement and excavation of materials being mined (Ono et al., 2016).The values of As concentrations found in all water samples are above the maximum permissible limit of both the World Health Organization, WHO (10 μgL^{-1}) and by the Mexican Norm, NOM-127-SSA1 (25 μgL^{-1}) (Monterrey; World Health, 2018). In this study area, the range of As concentration reported in water samples ranges from 98 to 163 μgL^{-1} (Loredo-Portales et al., 2017; Muñoz et al., 2016; Rodríguez et al., 2019). It should be noted that few studies have reported on the quantification of As in surface water from the Xichu area. Most studies focus on soil, sediment, and rock samples (Osuna-Martínez et al., 2020). It is important to mention that the main sources of water in this region are the river, streams and groundwater. Approximately 100 m from the mining area there are some active corn fields where this water is used for irrigation (Loredo-Portales et al., 2017), in addition to being used for domestic consumption.

3.2 Morphology and molecular identification of isolated strains. From the strains obtained, a total of 4 morphologically distinct bacteria capable of growing on As(III) and As(V) were selected (one from each sampling point) and isolated. The strains were named A1AJ, A1J, A4DJ and S3EJ. Figure 2 shows the morphological characteristics of the isolated strains. A1AJ, A1J and A4DJ were gram positive while S3EJ was gram-negative. For A1AJ, the colonies were yellow and rod-shaped. A1J presented a light pinkish-red color with a coccoidal ovoid shape. A4DJ had an orange color and a stick shape. In the case of S3-EJ, the colonies were circular, convex, mucoid and translucent beige. All strains were aerobic.

Figure 3 shows the phylogenetic tree of the 16S rRNA gene, where the relationship of the isolates is observed with support from the NIH genetic sequence database, GenBank® (Sayers, Cavanaugh, et al., 2021). Analysis of 16S rRNA sequence showed that A1J belongs to the *Rhodococcus gordoniae* W4937^T (99.34% identity) (Jones et al., 2004). The strain S3EJ showed 99.23% similarities with the *Pseudomonas kribbensis* 46-2^T (D. H. Chang et al., 2016). The strain A4DJ showed 100% similarities with several *Exiguobacterium,* but according to the phenotypic characteristics can presumably be identified as the *Exiguobacterium indicum* HHS 31^T (Chaturvedi & Shivaji, 2006). The strain A1AJ showed 99.78% similarities with the *Microbacterium hydrocarbonoxydans* NBRC 103074 ^{T} (Schippers et al., 2005).The summary of the molecular identification of the isolates is represented in Table 3. In this same context, based on the morphology observed in this study for each strain, there is an agreement with the resulted cited in other works (D.-H. Chang et al., 2016; Jones et al., 2004). It should be noted, that for the first time the molecular identification of native bacterial strains in this geographic region has been reported.

3.3 Minimum inhibitory concentration of the isolated. The As tolerance of the four bacterial cultures was tested by determining their minimum inhibitory concentration (MIC). The percentage analysis was in relation to the growth of the control group. The *R. gordoniae, E. indicum and M. hydrocarbonoxydans* were able to grow in 96 h under control conditions (absence of As) and in As up to 10 mM, both in media with arsenite (93.1 ± 7.2%) and arsenate (95.2 ± 4.4%). However, for the case of *R.gordoniae* in 12mM a slight growth of 32.5 % (As (III) and 39.8% (As (V)) was observed. For *E.indicum and M.hydrocarbonoxydans* average growth rates were of 4.3 ±0.8 % and 9.9 ± 3.1% in cell growth was observed in 12 mM As(III) and As(V) respectively. *P. kribbensis* presented a MIC of 5 mM (69.7%) for As(III), and 10 mM (88.4%) for As(V). The genus of these species has previously been

reported to be highly resistant to As (Kumari et al., 2019; Pandey & Bhatt, 2015). However, there are few related studies for *R. gordoniae, E. indicum, M. hydrocarbonoxydans* and *P. kribbensis*, specifically.

3.4 Effect of pH on the growth bacteria culture. The pH of the surrounding medium plays a critical role in determining As mobilization and toxicity and influences the occurrence and distribution of the microorganisms themselves. In microbial systems, pH controls the energy yields of redox reactions common in anoxic environments, including syntrophic oxidation, iron reduction, sulfate reduction, and methanogenesis(Cavalca et al., 2013). Figure 4 shows the results of the effect of pH on the growth of the four bacterial cultures. All bacterial cultures showed growth between pH 5.5 and 10. Very poor growth was observed within the pH range of 4.0 to 5.5. At acidic pH < 4, no growth was observed. The maximum OD_{600} value occurred for *R. gordoniae*, followed by *M. hydrocarbonoxydans, P. kribbensis and E, indicum,* at pH 8.5, 8.0, 7.5 and 7.5, respectively. Results from the present study are consistent with earlier reports where As-resistant bacteria were found to grow in wide range of pH 5–10 (Kumari et al., 2019) but were unable to tolerate extreme conditions, i.e. pH 2 or pH 11.

3.5. Gene expression analysis. In this work the presence of Aio genes encoding Aio enzyme (arsenite oxidase) by PCR was detected in: *M.hydrocarbonoxydans, P.kribbensis, E.indicum,* but not in *R. gordoniae,* Table 4. As with the results of previous work, the *Rhodococcus* strain failed to give positive amplification, probably due to mismatches between the tested primers and the gene sequence (Anna Corsini et al., 2014). However, it presented a significantly higher growth in terms of biomass generation and growth rate. The above suggests that *R. gordoniae* could metabolize arsenic in a chemoautotrophic manner. Previous studies have shown that species of the phylum Actinobacteria, Firmicutes, and Proteobacteria are distinguished by their ability to interact with inorganic arsenic (Hamood Altowayti et al., 2020). The genus *Rhocodccoccus* is reported with the presence of mostly reducing genes, but also the presence of As(III)-oxidizing genes (Kumari et al., 2019; Prasad et al., 2011; Wang et al., 2020). The determination of the presence of resistance-conferring genes is influenced by the environmental conditions to which they are exposed (Wang et al., 2020). On the other hand, the presence of arsenic in water serves as a selection of microorganisms and at the same time enriches their growth due to the presence of this operon. One example is that, in conditions with high sulfate-reduction activity, genes such as *aioA* and *aoxB* enriched by oxic environments are inhibited by this activity. In accordance with previous results (A. Corsini et al., 2014), these data corroborate the hypothesis that the ars operon could be slightly different even though the strains are categorized in the same genera, supporting the hypothesis of a horizontal transfer of the arsenic genes within bacterial population.

3.6 Comparison of the growth profile among the four bacteria under As(III) and As(V) stress. The growth patterns for *R. gordoniae, M. hydrocarbonoxydans, E. indicum* and *P. kribbensis* under arsenite and arsenate stress are shown in Figure 5. Based on the results obtained in the MIC, the As concentration used for this study was 10 mM for both As(III) and As(V).The assays were designed through the 8x4 factorial design, to evaluate the effect of strains and time on bacterial growth in the presence of As(III) and As(V). A value of $p<0.05$ was obtained for strain-time interactions, with an effect on their growth in the presence of As(V) and As(III). By means of Tukey's multiple comparisons analysis, the optimal growth times of the isolates in the presence of both inorganic As species and the significant differences in their behavior were identified, as well as the selection of the bacterial strains with the greatest capacity for interaction with As. *R. gordoniae* (*R.g*) was shown to be the strain with the highest growth capacity in the presence of As(III) and As(V), presenting a statistically higher growth compared to the other strains under study, its optimal growth times (exponential phase) being 144 h for As(V) and As(III). On the other hand, strain *M. hydrocarbonoxydans* (*M.h*) showed good growth although significantly ($P<0.05$) lower

than *R. g* for both As(III) and As(V). *M.h* showed constant growth up to 168 h in the case of As(V), while in the presence of As(III) the exponential phase occurred at up to 120 h. In the presence of As III, an interesting phenomenon was observed at 120-168 h by *M. hydrocarboxydans*. It showed a biphasic behavior, similar to a diauxic growth. Possibly due to the fact that within its detoxification mechanism, *M.h* presents an adaptive mechanism. As(III) as a stressful stimulus, it activates an adaptive response that increases the resistance of the bacterium to a higher level of stress. This response may involve the expression of genes encoding cytoprotective proteins, such as chaperones, heat shock proteins, antioxidant enzymes and growth factors. On the other hand, *E. indicum* (*E.i*) showed growth capacity in the presence of As(III) and As(V). However, its behavior is different; while in the presence of As(III) it presents significant ($P<0.05$) and continuous growth up to 96 h while in the presence of As(V) it presents exponential growth in 24 h and enters a stationary phase from 24 to 168 h without significant differences ($p\geq0.05$). For *P. kribbensis* (*P.k*), an effect on its growth was identified through interaction with As(V), reaching a maximum growth in 168 h, which in turn is statistically equal to *M. h,* while in the presence of As(III), it is completely inhibited. The growth of the isolates in the presence of As(III) and As(V) was presented as follows: from the highest growth capacity to the lowest, according to the Tukey test: a) As(III); *R.g>M. h>E.i>P.k*, and, b) As(V); *R.g>M.h=P.k>E. i.* For the cases mentioned *R.g* presents statistically higher growth in comparison with the other isolates under study after 24 h interaction, being the bacterium with the highest growth capacity with As(III) and As(V), and also at a higher rate. *M.h* presents good growth in the presence of As(III), with a metabolism probably differing from *R.g* because it is higher. *M.h* reached its optimum growth at 144 h, while for this average *R. g* reached it after 48 h. As reported in other previous works, the duration of the lag phase gradually increased as the As(III) concentration in the medium was increased, and as mentioned above this could be due to the fact that with increasing As(III) concentration, an adaptive phase was required to tide over high toxicity of As(III), (Kumari et al., 2019). In the presence of As(V), the correlation coefficient values for a 168 h growth period for *R.g, M.h, E. i and P. k* were -0.9935, -0.9792, -0.5884 and−0.9932, respectively. While in As(III) for *R.g, M.h,* and *E.i* they were -0.9966, -0.9911 and -0.9636, respectively. Negative values suggest that As stress has an inversely proportional effect on the growth of the bacteria (Kumari et al., 2019).

3.7. Removal of As(III) and As(V) by isolated strains. As in the previous section, the concentration of As in the medium was based on the result of the MIC, (10 mM was used for As(III) and As(V)). Based on the above results, in the case of *P. kribbensis*, it was analyzed for As(V) only. The pH used was 8.5 since this is the average pH present in the contaminated area of study. Maximum As-removal occurred during the initial growth phase, between 24h (*E. indicum*), 48 h (*R. gordoniae* and *M.hydrocarbonoxydans*) and 72h (*P. kribbensis*). In the same context, the concentrations were 2.52 mM (*E. indicum*), 3.19 mM (*M.hydrocarbonoxydans*) and 4.03 mM (*R. gordoniae*) for As(III) and 3.09 mM (*R. gordoniae*), 3.33 mM (*M.hydrocarbonoxydans*), 5.21mM (*E. indicum*) and 2.57mM (*P. kribbensis*) for As(V**).** The results of this study revealed different behaviors between the percentages of As(III) and As(V) removal by isolated strains, Figure 6. In the case of *E. indicum*, it was more efficient in the removal of As(V) than As(III). At the end of the experiment (168 h), *E.indicum* successfully removed approximately 74.83±1.2% of As(V) from the medium, while only 61.77±0.8% removal was achieved in the case of As(III), Figure 6(a). For this genus, as in previous studies, removal percentages of 99% for As (V) and 90%-99% for As (III) (Pandey & Bhatt, 2016a, 2016b) have been reported, depending on the method developed, Table 5. Bacteria of the genus *Exiguobacterium* are adapted to use multiple strategies that allow them to maintain themselves under the stress conditions they face in their environments, namely the high toxicity generated by As (Andreasen et al., 2018). For this, bacteria use a large arsenal of proteins related to protein synthesis, detoxification, energy generation, transport and global stress (Pandey & Bhatt, 2015).The differences between the

level of resistance exhibited by the isolated strains and the reported strains may have resulted from the variations in the levels of contamination, source of contamination, period of exposure, the characteristics of the environment and variations of metal or metalloid bioavailability (Aguilar et al., 2020). The cases of the strains belonging to the Actinobacteria phylum, *R. gordoniae* and *M.hydrocarbonoxydans* were slightly more efficient in the removal of As(III), 81.6 ±1.1% and 79.98±2.2% ; while for As(V) they were 77.21±0.9% and 68.93±2.8% , respectively, (Figure 6 (b, c)). For both genders, in comparison with previous work (A. Corsini et al., 2014; Pandey & Bhatt, 2016b; Prasad et al., 2011), the results obtained were within the reported range of 48.43% to greater than 90%, Table 5. The possible explanation between the removal capacity of As(III) and As(V) by *R. gordionae* and *M. hydrocarbonoxydans,* observed in this study, is that they can present systems capable of both reducing and oxidizing As. The transformation reactions are not only affected by the bacterial functional enzymes, but also the reduction/ oxidation potential in the environment, which is greatly influenced by the presence of microbial communities. There is information on As redox cycling by microorganisms which play a significant role in controlling As speciation and mobility in high As environments. In this study, it was observed that *P. kribbensis* was able to remove up to 80.23±0.3% of As(V), Figure 6(d). However, it presented limitations to removing As(III) (section 3.3). It is well documented that As toxicity depends on its chemical form, with inorganic forms being the most toxic. In most of the reported cases, the percentage of As taken up by bacteria is higher with As(V) due to it having lower toxicity than As(III) (Aguilar et al., 2020). Arsenate is taken up into the cell membrane by phosphate transporters, once As(V) binds to phosphate, the cell cannot detect its presence, it being highly accumulated in the cell. Moreover, this behavior is related to the fact that the bacteria can use the energy generated by As(V) reduction. It is worth mentioning that, this bacterium has been little studied, since it was recently discovered (D. H. Chang et al., 2016). Therefore, this is the first time that As removal studies by *P. kribbensis* have been presented.

3.8 Possible mechanisms that play a role in removal of As in isolated strain. By means of a bioinformatic analysis that was carried out on the bacteria identified in this work, it is considered that the detoxification of arsenic*, R. gordoniae* possibly uses the system operon ars, which transports arsenite out of the cell (A. Corsini et al., 2014). This operon includes a cytoplasmic arsenate reductase (arsC) that uses reduced glutathione (GSH) to convert arsenate to arsenite. ArsC forms an active complex with GSH, arsenate, and glutaredoxin (GRX). The final product, arsenite, is the substrate of the ArsA-ArsB efflux pump that renders the cells resistant to Asals. The 5-gene operon, arsRDABC, where appear the Ars A (ATPase) and Ars D (Chaperone), conferring resistance to higher As levels and exerting a tighter regulation (Cavalca et al., 2013; Saiki, 1990), both being in different replicons, chromosomal type. It should be noted that *R.gordoniae* does not have one ars operon, but 2 ars operons on its chromosome (RBC and RADBC), which is the reason this bacterium has a very high resistance to As. For *E.* indicum HHS 31 and *P. kribbensis* CHA-19, *Pseudomonas* generally have at least one arsRBC operon that contains the enzyme arsenate reductase referenced as ArsC that is part of a system for detoxifying arsenate. The substrate binds to a catalytic cysteine residue, forming a covalent thiolate-As(V) intermediate. A tertiary intermediate is then formed between the As, the enzyme's cysteine, and a glutathione cysteine. This intermediate is reduced by glutaredoxin, which forms a dithiol with the glutathione, leading to the dissociation of arsenite which can be extruded from some bacteria by arsenite-transporting ATPase. This System also contains Spx, a general regulator that exerts negative and positive control over transcription initiation by binding to the C-terminal domain of the alpha subunit of RNA polymerase (Cavalca et al., 2013). For the case of the detoxification mechanism detected in *M. hydrocarbonoxydans* SA35, Macur, et al (Macur et al., 2004) have reported that the mechanism of As(III) oxidation by *Microbacterium* is related to As detoxification rather than energy generation.

Arsenate is acquired by an organism through endogenous Pi (inorganic phosphate) transport systems. Inside the cell, arsenate is reduced to the thiol-reactive form arsenite. Glutathione (GSH)-conjugates of arsenite may be extruded from the cell or sequestered in vacuoles by members of the ATP-binding cassette (ABC) family of transporters, (LeBlanc et al., 2013). The isolation of both oxidizing and reducing strains from same species, shows that the ability to either oxidize As(III) or reduce As(V) is variable even among strains that proliferate under the same environmental conditions. Figure 7 shows the likely mechanism of microbial transformations of arsenic: a) In detoxification and energy generation, arsenite oxidase (Aio) is responsible for the oxidation of As(III), arsenite functions as an electron donor in the initiation of a membrane respiratory chain, b) In arsenate reduction, energy generation, arsenate reductase (Arr) participates in the reduction of As(V), arsenate functions as a terminal electron acceptor for an anaerobic respiratory chain, c)As(V) is taken up by organisms through phosphate transporters and As(III), being in an uncharged form, enters the cell through the aquaglyceroporin (GlpF) transporter. As(V) is then reduced to As(III) by bacterial ArsC. Glutathione and glutaredoxin serve as a source of reducing potential. Possibly, arsenite is extruded from cells only by ArsB, where energy is supplied by the cell membrane potential or by an arsenite translocating ATPase (ArsAB ATPase). The ArsA protein is an ATPase that interacts with ArsB to form the arsenite expulsion pump energized by ATP hydrolysis. The main function of Ars D is related to its ability to bind arsenite and transfer it to the ArsA ATPase, prior to oxyanion expulsion by the ArsB pump. In general terms, it is worth mentioning that in the analysis of arsenic removal by microorganisms, the stress mechanisms employed by the microorganisms for their survival and acclimatization in arsenic-rich environments must also be considered. In the present study, *R. gordoniae, M. hydrocarbonoxydans, E. indicum and P. kribbensis* were found to be able to tolerate arsenic, as well as remove arsenite and arsenate. The results of the present study indicate that native microorganisms could be good candidates for the field of in situ bioremediation. Future prospects for research in this field could include the sustainable application of molecular tools and technologies. Recent advances in genetic engineering, including techniques such as gene editing, systems biology and metabolic engineering and nanozyme based bioremediation provide further insight into microbial metabolism and could be employed to improve bioremediation capabilities.

Conclusions

The application of microorganisms in the field of treatment of water contaminated by metalloids or metals is immense as remediation using microbial ecological methods is emerging as a promising technology. The search for sustainable biological techniques has been gaining momentum in recent times, and it is necessary to carry out more research oriented in the search, application and treatment of contaminated water or contaminated sites by microbial application. Bacteria from As contaminated sites are able to tolerate high levels of this metalloid and are capable of removing it. The results of the present study indicate that native microorganisms could be good candidates for bioremediation. Of the four bacterial species investigated, *R. gordoniae* and *M. hydrocarbonoxydans* were adapted to a wider pH range and were able to tolerate higher As(III) and As(V) stress and could be chosen as potential candidates in bioremediation. However, the study of *E. indicum* and *P. kribbensis* also offers a potential field of research for As removal under optimal conditions.

Declarations

Compliance with ethical standards

Conflict of interest. The authors declare that they have no conflict of interest.

Ethical approval. This article does not contain any studies with humans or animals performed by any of the authors.

Acknowledgements

The authors would like to acknowledge the Consejo Nacional de Ciencia y Tecnología (CONACYT) and the University of Guanajuato (Engineering Division and Directorate for Research and Postgraduate Support).

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Tables

Table 1. *In-situ* physico-chemical characterization of water samples in dry season. Level and Standard Deviation (SD), n=3.

Parameters	Sampling points			
	AJ	J	EJ	DJ
SO_4^{2-} (mg L^{-1})	110±1.1	132.5±1.3	145±2.1	160±2.1
pH	8.1±0.1	9.1±0.2	8.5±0.9	9.2±1.5
S^{2-} (mg L^{-1})	n.d.	n.d.	n.d.	n.d.
C.E (µScm^{-1})	630±1.5	957±2.1	653±3.2	661±2.6
O.D (mg L^{-1})	5.7±0.9	4.68±0.1	4.18±0.6	4.45±0.1
T(°C)	28.0±1.8	29.5±0.4	29.6±1.6	27.8±1.2
Fe^{2+}(mg L^{-1})	0.04±0.01	0.036±0.01	0.042±1.6	0.08±0.01
NO_3^- (mg L^{-1})	9.6±1.8	1.25±1.02	6.8±2.1	0.9±0.01
K^+ (mg L^{-1})	1.8±0.1	1.9±0.22	1.29±0.14	2.9±0.33
Mg^{2+} (mg L^{-1})	33.2±1.88	38.1±1.92	30.9±1.01	32.01±2.42
Mn^{2+} (mg L^{-1})	0.27±0.03	0.38± 0.02	0.01±0.01	0.15± 0.02
Zn^{2+} (mg L^{-1})	0.012±0.01	0.045±0.01	0.035±0.001	0.027±0.01

n.d.= not detected

Table 2. Determination of Arsenic concentration (µg L^{-1}) in water samples by HG-AAS.

Water samples	**Sampling point**			
	AJ	J	EJ	DJ
	35.02 ± 0.2	40.5 ± 1.1	50.3 ± 2.9	108.1 ± 2.1
Analytical performance of HG-AAS method: LOD=0.019 µg L^{-1} (blank, n=20), R^2 =0.9997 (calibration curve), %RSD=3.1, Relative error = -2.9% (in relation to SMR).				

**Genes identified in this work

n.d: not detected

Table 3. Summary of the Molecular Identification.

Isolated strains	Closely related species	Acces numbers GenBank
1DA	*Rhodococcus gordoniae*	NR_025730.1
3DS	*Pseudomonas kribbensis*	KT321658.1
4DA	*Exiguobacterium indicum*	MT256271.1
13DB	*Microbacterium hydrocarbonoxydans*	MK424292.1

Table 4. Results of molecular identification.

Key	Genus	Phylum	Tinción Gram	Genotype*
1DA	*Rhodococcus*	Actinobacteria	positive	n.d
3DS	*Pseudomonas*	Proteobacteria	negative	ars C, aioA**
4DA	*Exiguobacterium*	Firmicutes	Positive	ars C, aioA**
13DB	*Microbacterium*	Actinobacteria	Positive	ars C, aioA**

**Genes identified in this work

n.d: not detected

Table 5. Summary of bioremediation efficiency of various bacterial species.

Bacteria	Source of isolation	Media used for As remediation	Method of remediation employed by bacteria	Remediation efficiency	Reference
Exiguobacterium sp.	Soil from Rajnandgaon, Chhattisgarh, India	Basal Salt Medium (BSM)	Biosorption	99% of As (V), 90% of As (III)	(Pandey & Bhatt, 2016b)
Exiguobacterium sp.	Soil from Chhattisgarh, India	Water samples	Immobilized in calcium alginate	99% of As (III)	(Pandey & Bhatt, 2015)
Exiguobacterium sp.	Subsurface soil from Chhattisgarh, India	Basal salt medium(BSM)	Biosorption, bio-oxidation	99% arsenic under aerobic conditions	(Pandey & Bhatt, 2016a)
Exiguobacterium profundum PT2	Wastewater samples were collected from the outer drain of an industrial paint plant, Kasur, Pakistan	Planktonic cultures and biofilms	Biosorption and biotransformation	25.2 and 29.4 mg g^{-1} biomass, respectively	(Andreasen et al., 2018)
Microbacterium paraoxydansis	Soil samples from districts of Chhattisgarh, India.	Basal salt medium (BSM)	Biosorption	87 ± 0.9% of As (III)	(Pandey & Keshavkant, 2019)
Microbacterium sp. CSA40	Generation of biogenic manganese oxides (BMnOx)	Groundwater	Biosorption, bio-oxidation	>90% of total As was removed by 0.5 g/L BMnOx	(Liang et al., 2017)
Pseudomonas genus, As7325	Sediment and groundwater from Southern Zhuoshui River alluvial fan of Taiwan	Artificial groundwater	Bio-oxidation	As7325 oxidized 15000µg l^{-1} As(III) to As(V)	(Kao et al., 2013)
Pseudomonas aeruginosa, AT-01	Groundwater from Pakistan	Luria Bertani broth supplemented	Biosorption	Remove As with 98% efficiency	(Tariq et al., 2019)
Rhodococcus sp. MTCC 4400	MTCC Chandigarh	Minimal media (with yeast	Biosorption, bioaccumulation accompanied	80.2% total As(III)	(Kumari et al., 2019)

	(India)	Extract)	with bio-oxidation	removed in 12 h for 500 µM	
		Artificial groundwater		48.43% total As(III) removed in 6 h for 500 ppb	
Rhodococcus sp.	Subsurface water sample from contaminated site of West Bengal, India	Batch scale by adding a different amount of sorbent (Biomass)	Biosorption	77.3 mg/g of As(III)	(Prasad et al., 2011)
Rhodococcus sp. strain 6G	Water samples from the Province of Cremona, Lombardia, Italy	BBWM culture medium	Bio-oxidation	75 mgL^{-1} As(III) in 48 h,	(Anna Corsini et al., 2014)
Exiguobacterium indicum	Water samples from the Xichu River (mining area), Guanajuato, Mexico	Artificial water (Based on the analysis of the Xichu river)	Biosorption, bioaccumulation	61.77 % of As(III), 74.83% of As(V)	**This study**
Microbacterium hydrocarbonoxydans				79.98% of As(III), 68.93% of As(V)	**This study**
Rhodococcus gordoniae				81.6 % of As(III), 77.21% of As(V)	**This study**
Pseudomonas kribbensis				80.23% of As(V)	**This study**

Figures

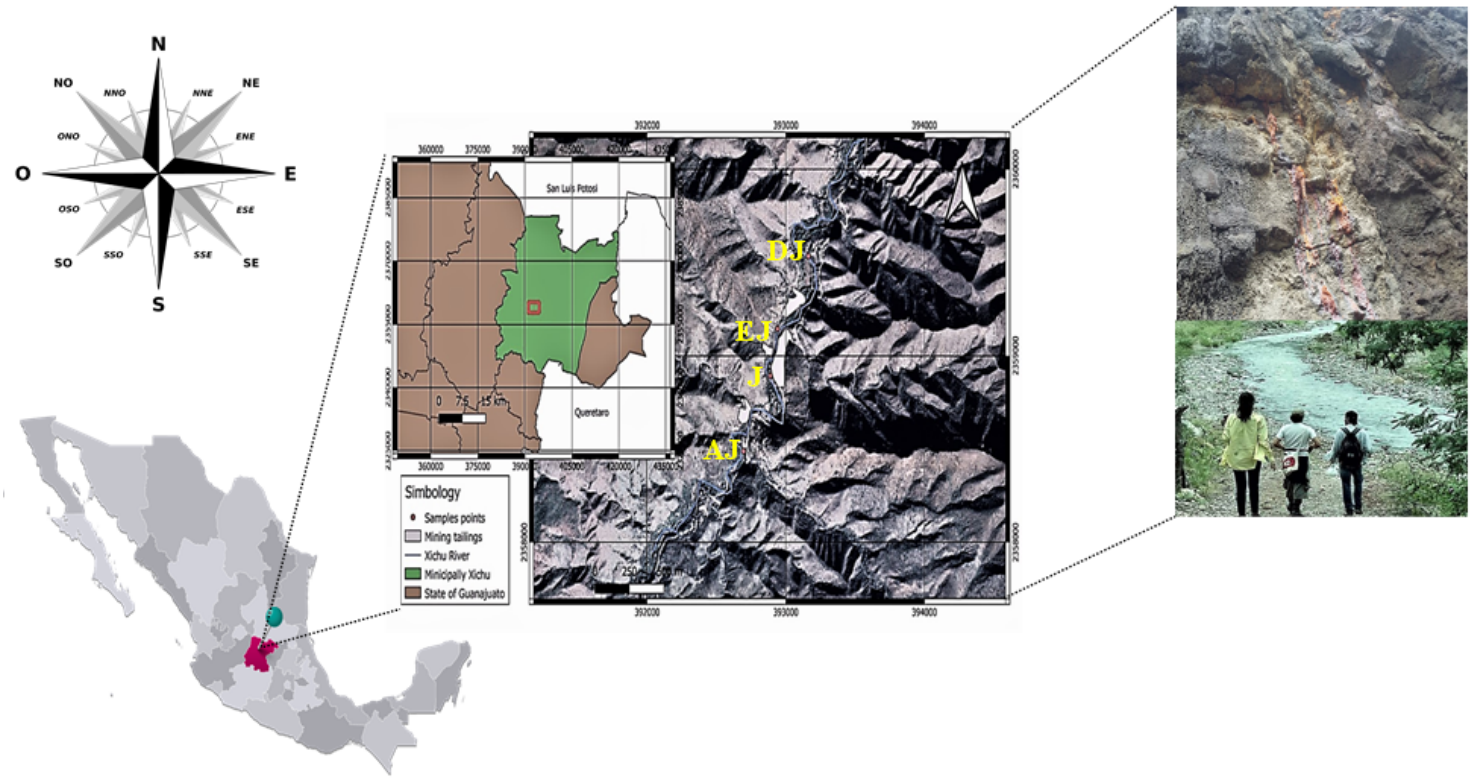
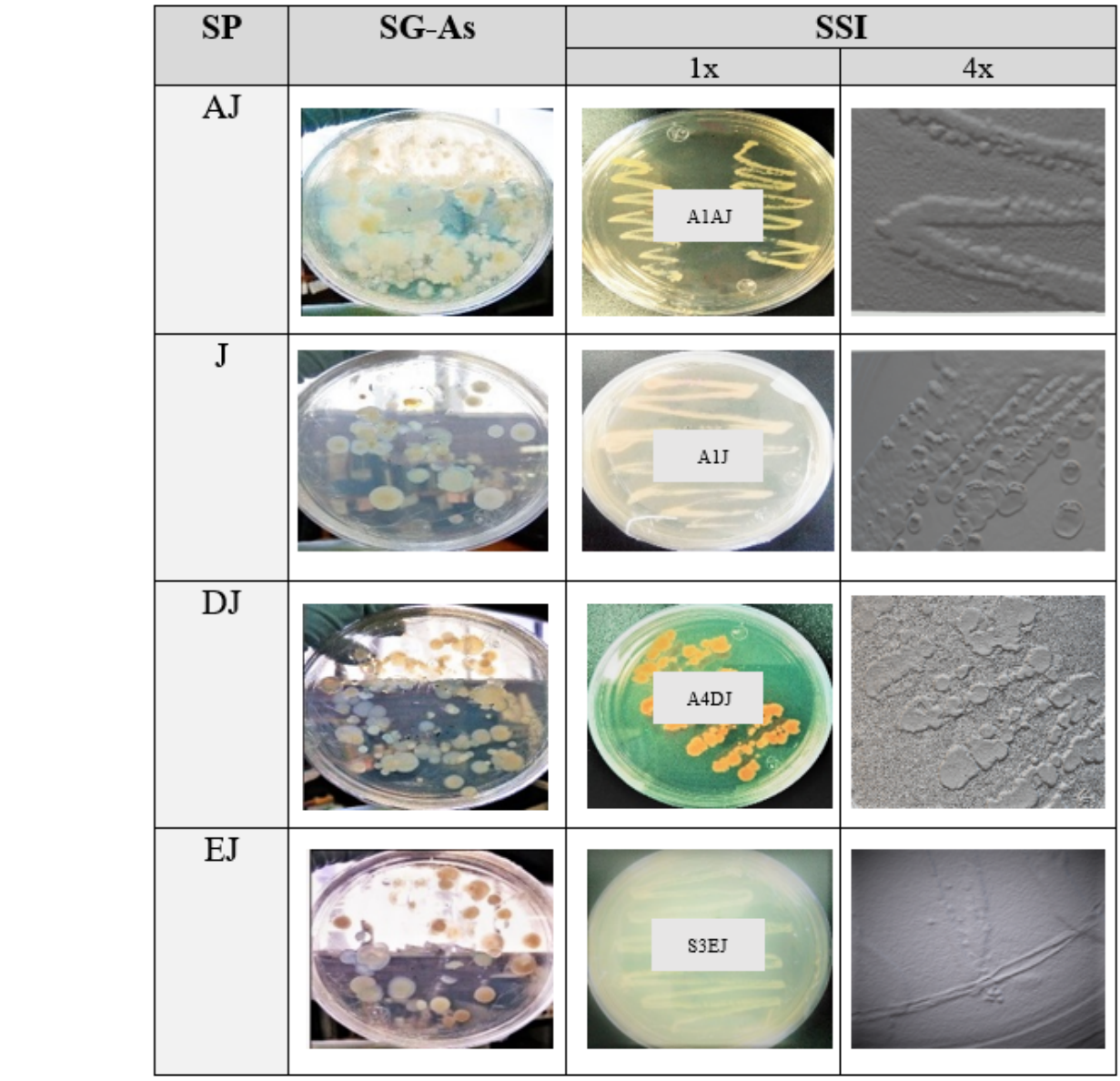


Figure 1

Sampling site, Xichu River, Guanajuato, Mexico. The location points were AJ (21°19´40.04´´N,100°01´57.18´´O), EJ (21°19 52.88” N, 100°01´54.09´´O), J (21°19´48.13´´N, 100°01´57.89´´O) and DJ (21°19´57.81´´N, 100°01´48.87´´O). The map was created by QGIS 3.14 software. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.



SP: Sampling point
SG-As: Strains grown on arsenic-containing media
SSI: Strain selected and isolated

Figure 2

Native strains obtained at the different sampling points. The second column shows the strains that grew in culture media with the presence of arsenic. The third and fourth columns show the growth of the isolated native strains, where the color of the strain (1x, by optical microscopy) and the shape (4x, by 3dp.rocks/lithophane software) can be appreciated.

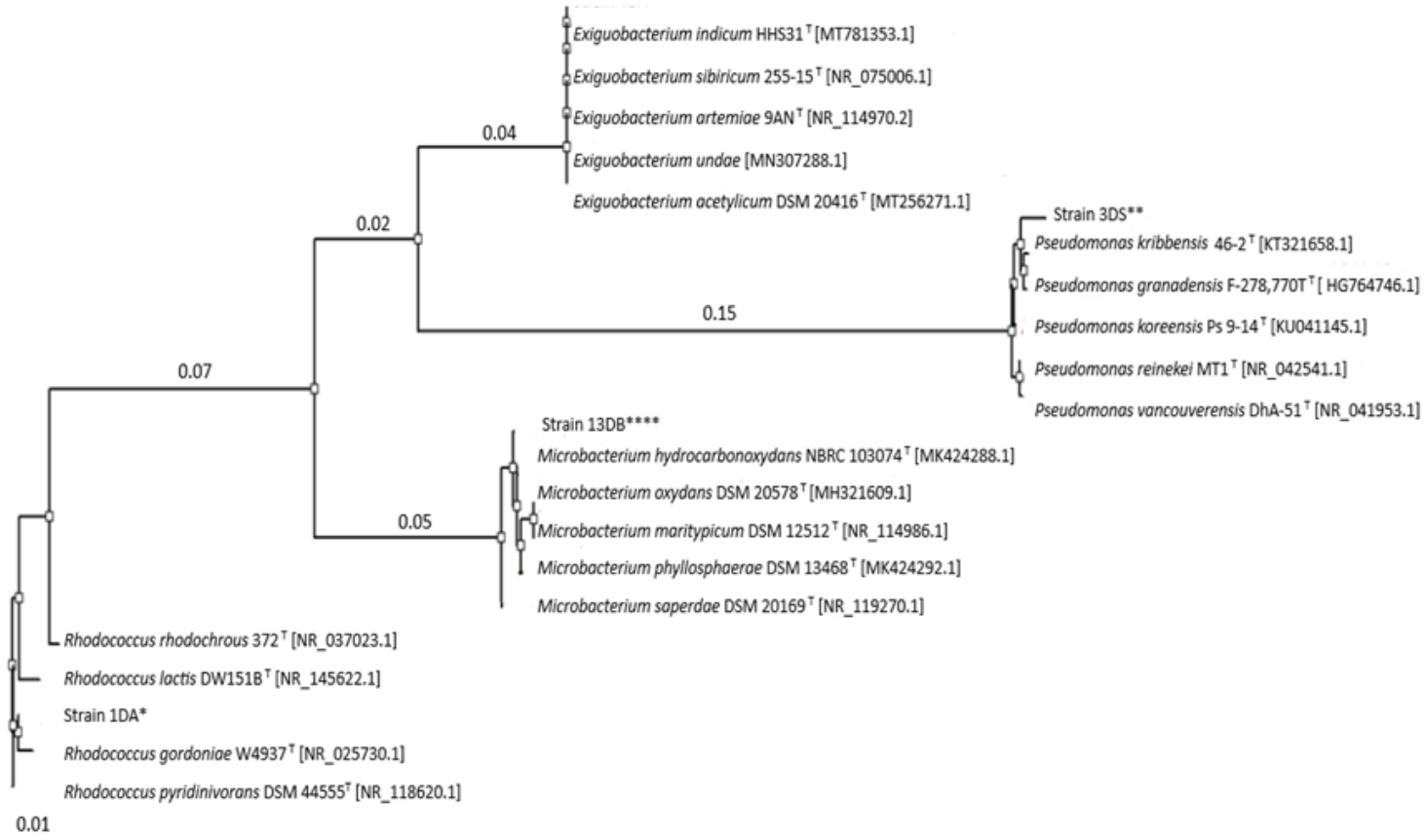


Figure 3

16S rRNA sequence based phylogenetic tree for the strains: 1DA*, 3DS**, 4DA*** and 13DB**** compared to other bacteria in GenBank® by using the neighbor-joining method. Asterisks indicate our isolates.

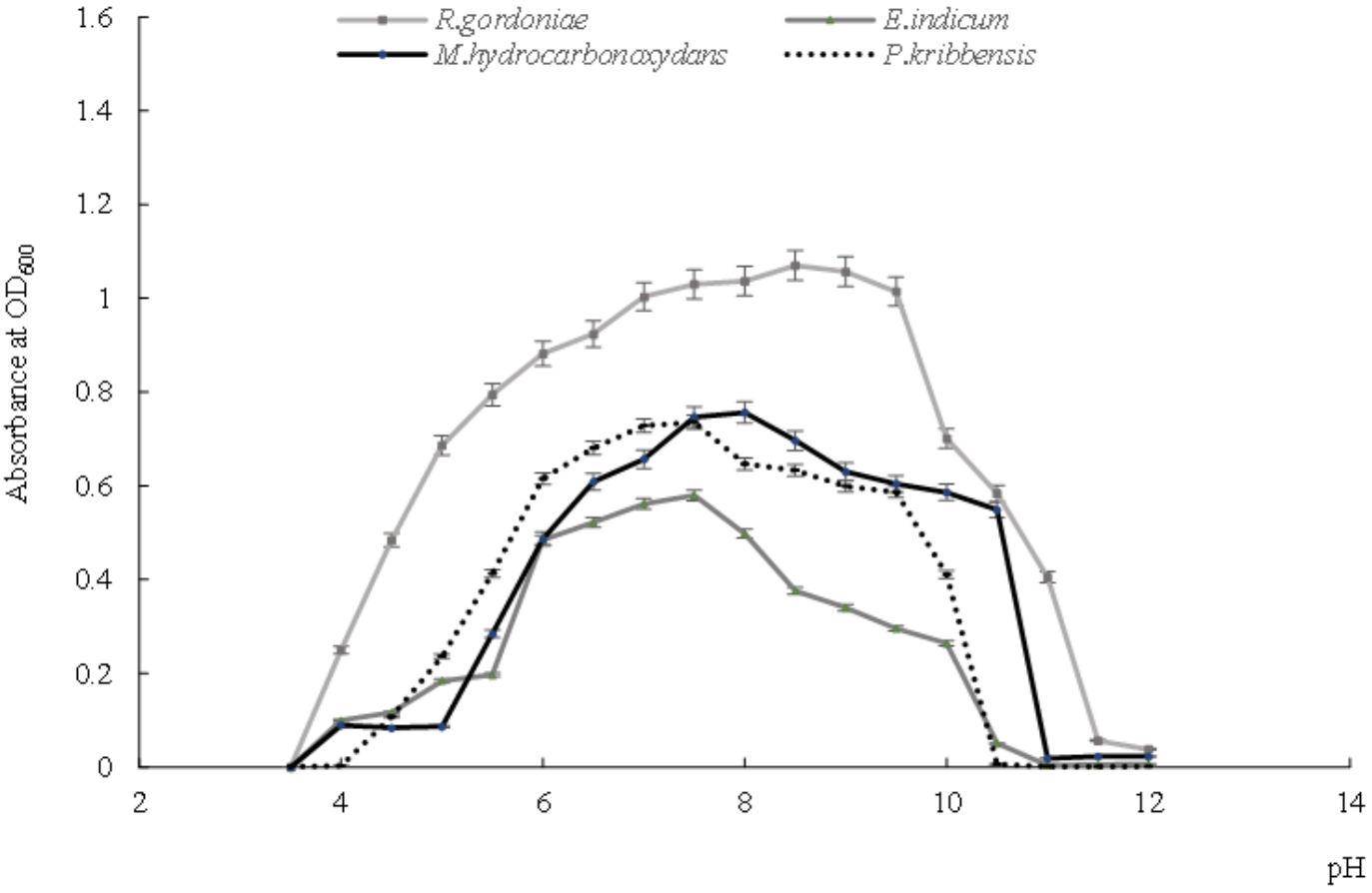


Figure 4

Growth of R.gordoniae, M.hydrocarbonoxydans, E.indicum and P.kribbensis under pH ranging from 3.5 to 12. Change in OD600 of culture was measured over 24 h (error bars indicate the standard error of triplicate samples).

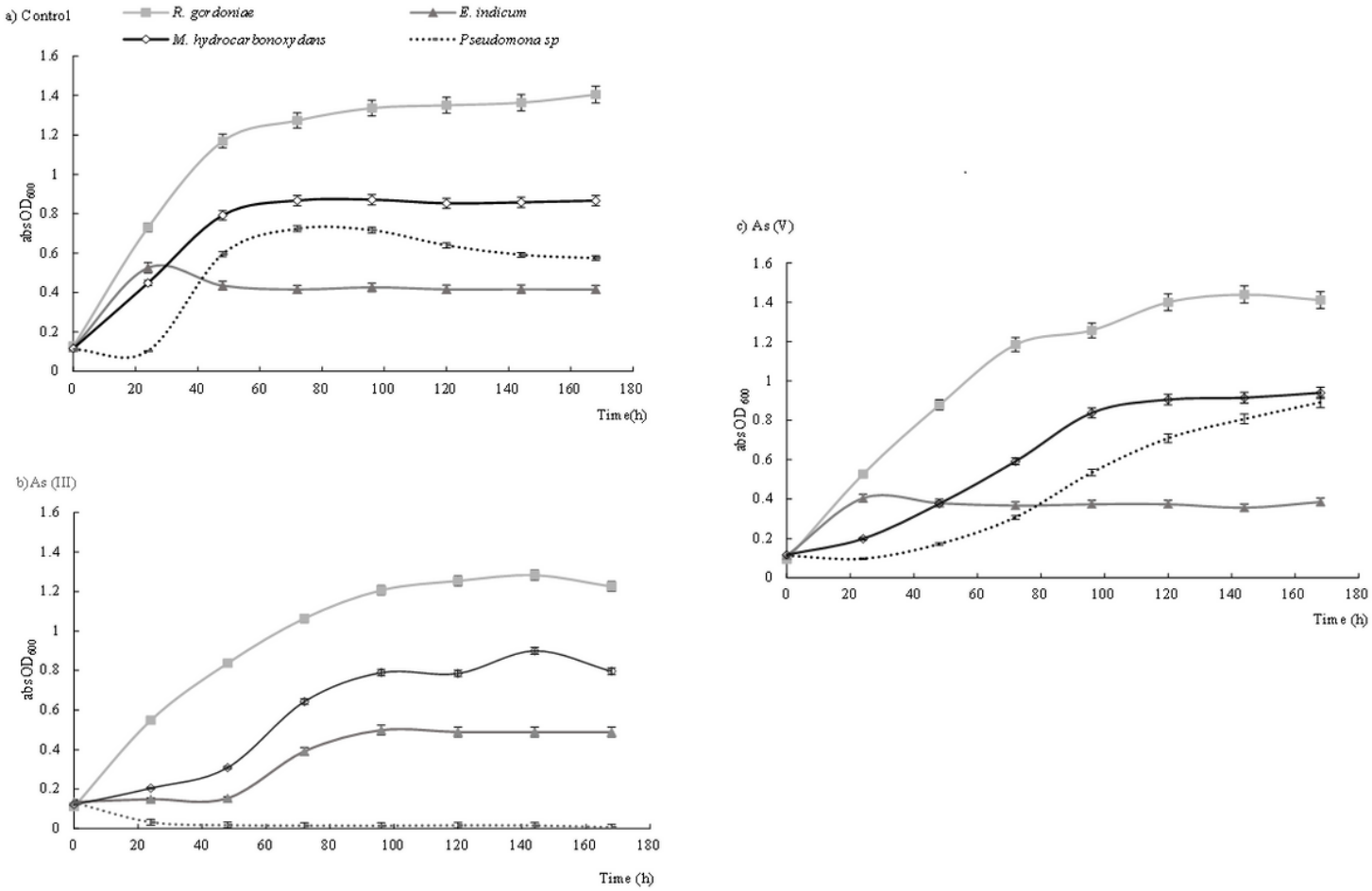


Figure 5

Growth of R.gordoniae, M. hydrocarbonoxydans, E.indicum and P. kribbensis, under concentration of inorganic arsenic species, 10 mM. a) Control, b) As(III) and c) As (V). Error bars indicate the standard error of the mean of three experiments.

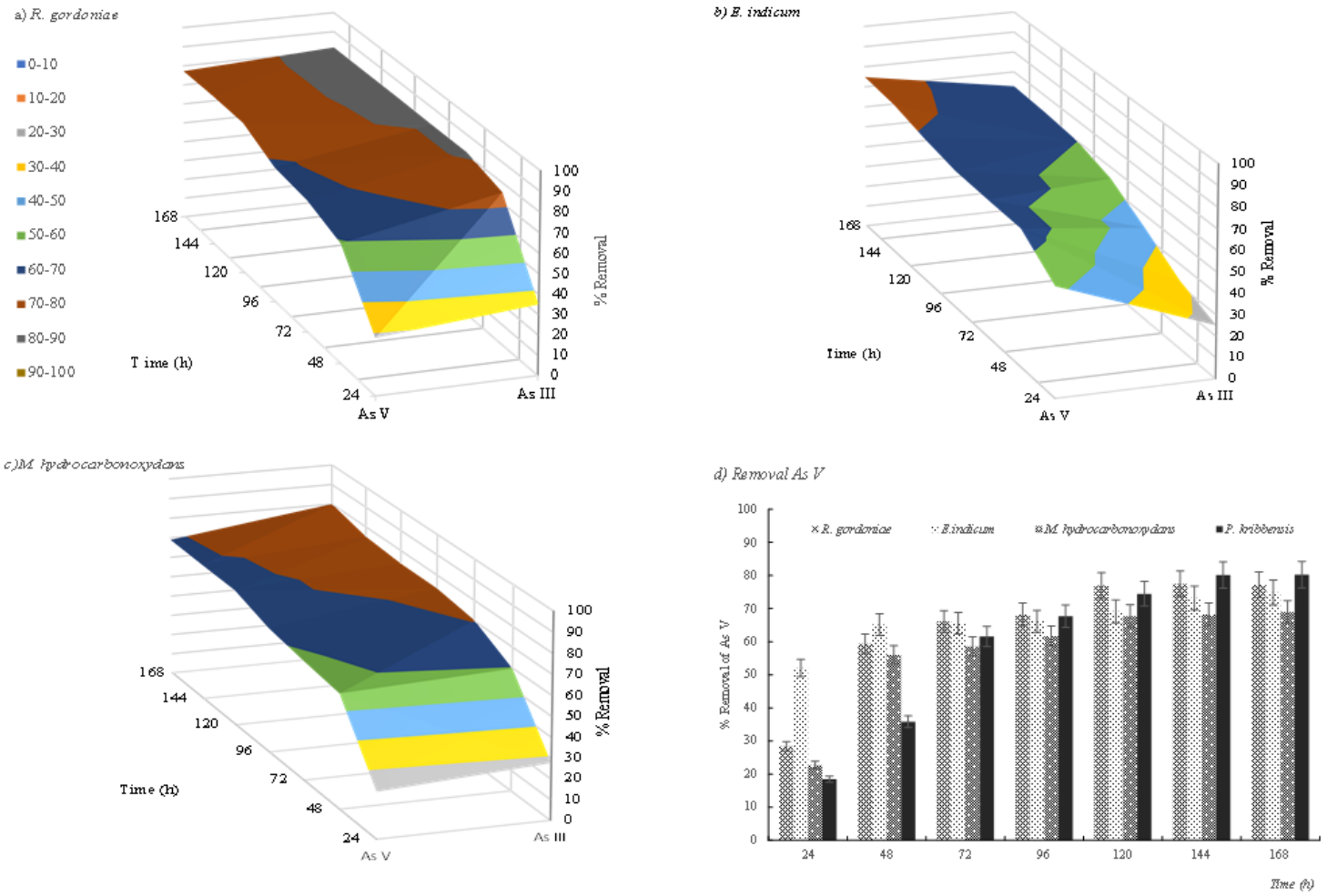


Figure 6

Graphics of the percentage removal of As(III) and As (V) at 24 h intervals for 168 h. a) R.gordoniae, b) E. indicum, c) M. hydrocarbonoxydans. d) Comparison plot of % removal for As (V) between P. kribbensis and the other three strains. In P.kribbensis no removal capacity was observed for As (III) at 10 mM. For graph d), the error bars indicate the standard error of the triplicate samples.

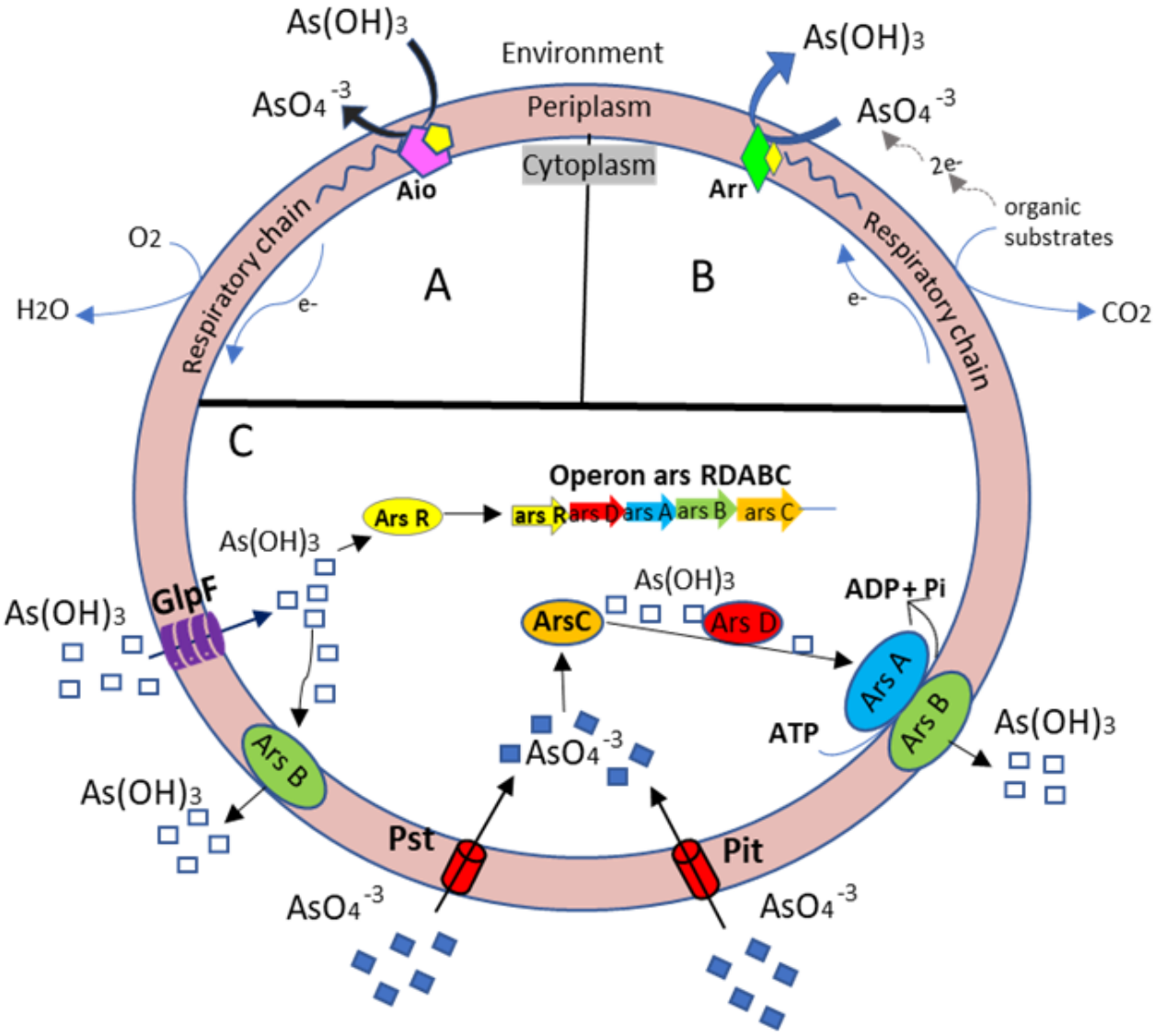


Figure 7

Mechanism of arsenic detoxification in prokaryotic cells. A) Arsenite oxidation: detoxification and energy generation; B) Arsenate reduction: energy generation; C) Mechanism of arsenic resistance: Arsenate (AsO4-3) is taken up by phosphate transporters (Pit and Pst), and arsenite (As(OH)3) is taken up by glycerol facilitator (GlpF). The arsenite binds to the ArsR repressor, inducing expression of the operon arsRDABC, which results in arsenate reduction and arsenite extrusion from the cells.