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Microbiomes in agricultural and mining soils contaminated with arsenic in Guanajuato, Mexico

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Abstract

In this report, physical and chemical properties, and total arsenic (As) concentrations were analyzed in agricultural (MASE) and mining soils (SMI) in the State of Guanajuato, México. Additionally, a metagenomic analysis of both types of soils was the bases for the identification and selection of bacteria and fungi resistant to As. The SMI soil showed higher concentration of As (39 mg kg^{-1}) as compared to MASE soil (15 mg kg^{-1}). The metagenome showed a total of 175,240 reads from both soils. MASE soil showed higher diversity of bacteria, while the SMI soil showed higher diversity of fungi. 16S rRNA analysis showed that the phylum Proteobacteria showed the highest proportion (39.6% in MASE and 36.4% in SMI) and Acidobacteria was the second most representative (24.2% in SMI and 11.6% in MASE). 18S rRNA analysis, showed that the phylum Glomeromycota was found only in the SMI soils (11.6%), while Ascomycota was the most abundant, followed by Basidiomycota, and Zygomycota, in both soils. Genera *Bacillus* and *Penicillium* were able to grow in As concentrations as high as 5 and 10 mM, reduced As (V) to As (III), and removed As at 9.8% and 12.1% rates, respectively. When *aoxB*, *arsB, ACR3(1), ACR3(2,)* and *arrA* genes were explored, only the *arsB* gene was identified in *Bacillus* sp., *B. simplex*, and *B. megaterium*. In general, SMI soils showed more microorganisms resistant to As than MASE soils. Bacteria and fungi selected in this work may show potential to be used as bioremediation agents in As contaminated soils.

Keywords Agricultural soil · Mining soil · Arsenic · Bacteria · Biodiversity · Fungi

Introduction

In the State of Guanajuato, Mexico, the agricultural land occupies 969,000 ha that generate 8.69 million tons, mainly of barley, corn, sorghum, alfalfa, broccoli, and wheat. This represents a market of approximately 42 billion USD and Guanajuato occupies the ninth position in Mexican agricultural production (http://www.inegi.org.mx/temas/agricultura/). However, economic development associated to population growth has caused a serious impact on soil resources.

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The state of Guanajuato has a mining exploitation history since the XVI century (mostly for Au and Ag extraction). Carrillo-Chávez et al. (2003) indicated that historic mine tailings are dispersed around the Guanajuato city and widespread in the hydrological basin, but nowadays they are mostly covered by vegetation, urbanization or dispersed by erosion.

Arsenic (As) pollution in the State of Guanajuato has gained more attention in the last years. Several studies performed in the region indicated high contents of As in groundwaters used for irrigation, which are above the accepted world standard limits, with a mean of 0.33 mg L^{-1} (Rodríguez et al. 2016), an accumulation in soils with a range of 1.0–14.0 mg/kg (López-Pérez et al. 2017; Zanor et al. 2019) and in crops with a mean of 14.1 mg kg^{-1} in barley (Saldaña-Robles et al. 2018), as As can accumulate in soil and be transferred into the food chain (Lu et al. 2010).

As is a trace element present in different ecosystems. Average As concentration in world-wide soils ranges from 0.2 to 40 mg kg^{-1} (Bundschuh et al. 2008). Chronic exposure to As represents a risk to the environment and human health, because it has been classified as one of the most toxic and carcinogenic metalloids known (Gu et al. 2018). This metalloid is mainly found in soil as its inorganic forms (arsenite, As (III) and arsenate, As (V)), and it can be transformed by redox processes to an organic form. Some microorganisms play a fundamental role in the incorporation of As in their metabolism through oxide-reduction reactions, enzymatic transformation, methylation, chelation, exclusion, and immobilization (Cai et al. 2009; Rangel-Montoya et al. 2015).

Being the microorganisms the most active elements of soil, they can respond quickly to anthropogenic pressures, and can become indicators of soil health and quality. Studies involved in the composition of the soil microbial community help to explore the potential risks associated to its pollution and provide information on possible remediation strategies (Feng et al. 2018). For example, the bacterium *Bacillus licheniformis* (strain DAS1) is able to remove 100% of As (V) at a concentration of 3 mM, with the release of 42% of As (III) into the culture medium (Tripti et al. 2014). Fungi of the genera *Rhizopus*, *Neocosmospora*, *Penicillium*, and *Aspergillus* isolated from soils contaminated with As (9.45–15.63 mg kg^{-1}), were able to remove between 10.92 and 61.58% of the metalloid (Srivastava et al. 2011). An initial step in As metabolism is the enzymatic reduction of As (V) to As (III) by the arsenate reductase (*ArsC* in prokaryotes and *Acr2p* in eukaryotes) (Mukhopadhyay and Rosen 2002). Genes coding for arsenite transport proteins, such as *arsB*, *ACR3(1)*, and *ACR3(2)* are found in bacteria resistant to As. These genes are commonly found in bacterial isolates from soil highly contaminated with As (Cai et al. 2009).

Detailed studies on microbial communities in soils that control the biogeochemical cycle of As in natural systems are challenging due to the extreme diversity of the microbiome and the fact that not all microorganisms can be isolated in the laboratory. Metagenomics offer an unprecedented opportunity to examine the adaptation of microbial communities to environmental toxicity factors (Das et al. 2017). In this work, the microbial community, at the metagenomic level, is reported to obtain a broad description of the microbial diversity in soils of Guanajuato, Mexico for the first time. Due to their heavily and historical anthropogenic use, two types of soil with high As content were studied: one of agricultural origin (MASE) and one of mining origin (SMI). As-resistant bacteria and fungi were identified and characterized for their ability to transform As. This information is relevant not only to know As-resistant microorganisms, but it gives a clear representation of the microbial diversity in highly different soils.

Materials and methods

Soil sample collection

A total 15 agricultural soil samples were obtained at the following location (UTM units): 2,285,086.96 m N by 265,924.63 m E, at 1726 m a.s.l., which subsequently were pooled to form a single sample (here on called MASE), close by to the city of Irapuato, in an asparagus crop field. In this area, soils are classified as vertisols, with high content of clay minerals (smectite group), a good water retention, and high content of organic matter. In the case of soils of mining origin, a total of 5 sub-samples were taken at the following location: 2,324,859.63 m N by 264,345.89 m E, at 1995 m a.s.l., within the perimeter of the city of Guanajuato. Sub-samples were pooled, too, here on called SMI sample. MASE and SMI soil samples were previously characterized (López-Pérez et al. 2017). Soils were sampled following the Mexican norms: NOM-021-SEMARNAT-2000 (DOF 2002), NMX-AA-132-SCFI-2006 (SCFI 2006) and NOM-147-SEMARNAT/SSA1-2004 (DOF 2007), and stored at − 80 °C for further use.

Physical and chemical analyses of soils

The physical and chemical characterization of MASE and SMI soils were analyzed by triplicate following the methodology required by the Mexican Norm NOM-021-SEMARNAT-2000 (DOF 2002). The physical and chemical properties determined were moisture (M), textural class (T), bulk density (BD), real density (RD), total porosity (TP), pH, organic content (OC), total nitrogen (TN), and C/N ratio. Total concentration of As was determined according to the

method 6010C recommended by EPA (2001), using Optical Emission Spectrometry with Inductively Coupled Plasma Thermo iCAP 6500 Duo. The geoaccumulation index (Igeo) of soil samples was analyzed based on the index proposed elsewhere (Müller 1969; Loska et al. 2004), to evaluate the level of As contamination. Comparison of variables from soil samples was analyzed by Tukey's honestly significant difference (HDS) method, using the Statgraphics Centurion XV.II software.

Total DNA extraction from soils and metagenomic sequencing

Total DNA was extracted from three 0.3 g sub-samples, of each soil type using the MoBio PowerSoil® DNA Isolation Kit as described by the manufacturer (Fierer et al. 2012). Purified DNA was sent to Macrogen Inc., (Korea) for sequencing and analysis. Primers 27F, 518R, and ITS1-F, ITS4-R (Table 1) were used to amplify the 16S rDNA gene and the internally transcribed spacers (ITS region), from bacteria and fungi, respectively. Data processing was performed using the Roche 454 GS FLX (3.0 v) software (http://technical-support.roche.com). DNA was pre-processed and clustered as follows: (1) short reads were filtered out and extra-long tails were trimmed. Filtered reads were clustered at 100% identity using CD-HIT-DUP (http://weizhongli-lab.org/cd-hit-otu/); (2) chimeric reads were identified and secondary clusters were recruited into primary clusters; (3) noise sequences in clusters were removed and the remaining representative reads from non-chimeric clusters were clustered using a greedy algorithm into operational taxonomic units (OTUs) at a user-specified OTU cutoff (97% ID at species level). Taxonomic assignment and microbiome diversity were analyzed as follows: (1) representative sequences were used from each OTU to assign its taxonomy, using the software QIIME-UCLUST (http://qiime.org). Sample libraries were downloaded from the service provider (Macrogene); and (2) to verify significant differences between community structures (bacteria vs. fungi), the community richness and diversity (Alpha diversity) was estimated on each sample by the service provider.

Isolation of As-resistant microorganisms

MASE and SMI samples supplemented or not supplemented with arsenic salts ($NaAsO_2$, $HAsNa_2O_4$) at 5 mM were serially diluted under sterile conditions, and plated on EXSC medium (500 g L^{-1} sterilized and filtered soil, 1 g L^{-1} yeast extract, 2 g L^{-1} glucose, 18 g L^{-1} bacteriological agar), incubating for 24–48 h (bacteria) or for 72 h or more (fungi) at 28 ± 2 °C.

Detection of the As oxidation–reduction

Microorganisms (bacteria or fungi) selected as previously described, were grown in EXSC medium supplemented with arsenic salts during 48 h (bacteria) or 4–6 days (fungi) at 28 ± 2 °C. Once colonies were observed, a solution of 0.1 M $AgNO_3$ was used to cover the plates. Development of brownish-red or yellow color indicates the presence of As(V) or As (III), respectively (Simeonova et al. 2004).

Table 1 Primers used to amplify different sequences for the identification of bacteria, fungi, and genes related to As transformations

Gene	Primer	Sequence (5′–3′)	References
16S rDNA	27F	GAGTTTGATCMTGGCTCAG	Yoo et al. (2016)
	518R	WTTACCGCGGCTGCTGG	
ITS	1F	CTTGGTCATTTAGAGGAAGTAA	Gardes and Bruns (1993)
	4R	TCCTCCGCTTATTGATATGC	White et al. (1990)
16S rDNA	fD1	CAGAGTTTGATCCTGGCTCAG	Weisburg et al. (1991)
	rP2	ACGGCTACCTTGTTACGACTT	
ITS	ITS5-F	GGAAGTAAAAGTCGTAACAAGG	Habtegebriel et al. (2016)
	ITS4-R	TCCTCCGCTTATTGATATGC	
aoxB	1F	GTSGGBTGYGGMTAYCABGYCTA	Inskeep et al. (2007)
	1R	TTGTASGCBGGNCGRTTRTGRAT	
arsB	darsB1F	GGTGTGGAACATCGTCTGGAAYGCNAC	Achour et al. (2007)
	darsB1R	CAGGCCGTACACCACCAGRTACATNCC	
ACR3(1)	dacr1F	GCCATCGGCCTGATCGTNATGATGTAYCC	Achour et al. (2007)
	dacr1R	CGGCGATGGCCAGCTCYAAYTTYTT	
ACR3(2)	dacr5F	TGATCTGGGTCATGATCTTCCCVATGMTGVT	Achour et al. (2007)
	dacr4R	CGGCCACGGCCAGYTCRAARAARTT	
arrA	CVF1	CACAGCGCCATCTGCGCCGA	Mirza et al. (2017)
	CVR1	CCGACGAACTCCYTGYTCCA	

Molecular identification of As-resistant bacteria

Bacterial DNA was extracted from selected single colonies as described earlier (Shuhaimi et al. 2001). This DNA was used as template to amplify the 16S rRNA gene, using primers shown in Table 1. The PCR conditions used were: 95 °C for 5 min, 30 cycles 95 °C for 40 s, 50 to 60 °C for 1 min (depending on the template DNA), 72 °C for 1 min, and a final amplification at 72 °C for 10 min. Amplicons were analyzed by 1% agarose gel electrophoresis and visualized in a Bio-Rad Gel Doc EZ Imager. The amplified 16S rDNA were sequenced using the Pyrosequencing method by Macrogen Inc., (Korea), and were assembled using the Seqman software (version 3.0). Once the contigs were obtained, they were subjected to a BLASTn analysis, using the GenBank database (Wang et al. 2007).

Molecular identification of As-resistant fungi

Fungal DNA was extracted according to Cruz-Avalos et al. (2019). The DNA was used as a template to amplify the ITSs using primers shown in Table 1. PCR reactions were carried out as follows: 94 °C for 4 min, 30 cycles of 95 °C for 40 s, 50 to 65 °C for 1 min (depending on the template DNA), 72 °C for 40 s, and a final step at 72 °C for 10 min (Habtegebriel et al. 2016). Amplicons were analyzed by 1% agarose gel electrophoresis and visualized in a Bio Rad Gel Doc EZ Imager. Amplified ITSs were sequenced using the Pyrosequencing method by Macrogen Inc., (Korea) and analyzed in the same way as bacterial sequences.

Identification of genes involved in the transformation of As

The identification of *aoxB*, *arsB, ACR3(1), ACR3(2)* and *arrA* genes in the bacterial isolates was carried out using the primers described in Table 1, and according to conditions reported previously (Achour et al. 2007; Inskeep et al. 2007; Mirza et al. 2017). Amplicons were sequenced by Pyrosequencing at Macrogen Inc., (Korea) and analyzed in the same way as bacterial sequences.

Quantification of As in culture media

Quantification of total As in the supernatant of selected microorganisms in culture medium was determined by Atomic Absorption Spectrometry, using the Thermo Scientific™ iCETM 3000 Series by flame. Samples were diluted 1:10 for the quantification, and a standard curve was obtained with the following concentrations of As (V): 10, 20, 30, 40 and 50 ppm. All analyses were performed by triplicate and the values were calculated in mM concentrations. The standard deviations were calculated using the Statgraphics Centurion XV.II software.

Results

Soil physical and chemical parameters

Physicochemical properties of both soil sample types were determined, and estimated parameters are shown in Table 2. The MASE soil presented higher moisture (6.74%) as compared to the SMI soil (1.47%). Statistical difference was observed in the BD values, despite both types of soils ranged between 1.00 and 1.19 g cm^{-3}, which are typical values for soils rich in clay minerals with clayed texture, and good infiltration and aeration capacity (Sumner 1999). According to the Mexican norm NOM-021-SEMARNAT-2000, pH in the MASE soil was moderately acidic (pH = 6.4). Conversely, in SMI soil pH was moderately alkaline (pH 8.1). TN content reached a value of 0.38% for MASE, which was significantly different to the value of 0.17% for SMI. Something similar was observed with C/N ratios as a higher value was recorded for SMI (25.69), as compared with MASE soil (11.86).

According to the Mexican standard NOM-147-SEMARNAT/SSA1-2004, total As content in MASE soil (15 mg kg^{-1}) and in SMI soil (39 mg kg^{-1}) were below and above the limit value, respectively, for soils used in agriculture, urbanization and commerce (22 mg kg^{-1}). According to Igeo values, MASE soil corresponds to the category of "moderately contaminated" (level 2) by As, while the SMI soil belongs to the category of "moderate to heavily contaminated" (level 3).

Assembly of metagenomic reads

The number of reads obtained from the sequencing of the MASE and SMI soils was 175,240, with approximately 76 million bp and an average reading length of 434 bp. A total of 7141 sequences from the 16S rRNA gene were aligned to the SILVA database (Quast et al. 2013) and 34,059 sequences from the 18S rRNA gene of both samples were aligned to the NCBI database (Benson et al. 2007). The sequences recognized a total of 254 operational taxonomic units (OTUs) with the 16S rRNA gene for MASE and 206 OTUs for SMI; while for the 18S rRNA gene, 98 and 110 OTUs were obtained for each soil, respectively.

The MASE soils presented a higher bacterial diversity than SMI soil as the Shannon index was estimated at 7.06 and 6.12, respectively, while the SMI soil presented a high fungal diversity than MASE soil with Shannon's indices estimated at 4.10 and 3.30, respectively. It is considered that values lower than two represent low diversity, and values

Table 2 Physicochemical properties in MASE and SMI soil samples

Sample	Texture class	Sand (%)	Loam (%)	Clay (%)	M (%)	BD (g cm^{-3})	RD (g cm^{-3})	TP(%)	pH	OC (%)	TN (%)	C/N	As (mg kg^{-1})	Class of I_{geo}
MASE	Clay	13.64 a	25.6 a	60.76 a	6.74 a	1.07 a	1.97 a	45.50 a	6.40 a	8.41 a	0.38 a	11.86 a	15	2
SMI	Loam	39.03 b	34.19 a	26.79 b	1.47 b	1.00 b	2.30 b	56.72 b	8.10 b	7.14 b	0.17 b	25.69 b	39	3

Means ($n=3$) for the variables are shown. Values with different letters are statistically different ($p<0.05$)

MASE agricultural soil, *SMI* mining soil, *M* moisture, *BD* bulk density, *RD* real density, *TP* total porosity, *OC* organic content, *TN* total nitrogen, *C/N* carbon–nitrogen ratio

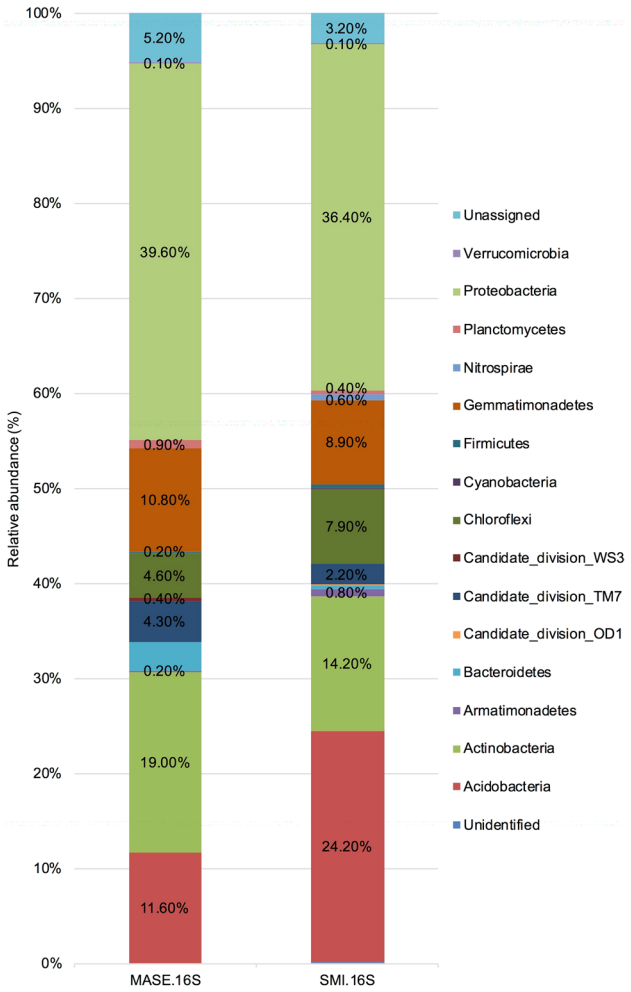

Fig. 1 Relative abundance at phylum level of bacteria in soil samples. MASE.16S: DNA samples from agricultural soil analyzed with the 16S rDNA gene sequences; SMI.16S: DNA samples from mine tailings soil analyzed with the 16S rDNA gene sequences

greater than three represent high diversity (Shannon 1948; Mora-Donjuán et al. 2017). According to the Simpson index, both soils showed dominance in the number of bacterial species, as this value is close to the unit (MASE = 0.98, SMI = 0.95), whereas the estimated index for fungal species showed a lower dominance of species (MASE = 0.69, SMI = 0.85) (Simpson 1949).

Taxonomic composition of the microbial communities

In terms of bacterial composition of the microbiomes, Proteobacteria, Acidobacteria, and Actinobacteria were the dominant phyla in MASE and SMI soils, representing more than 72% of the total population (Fig. 1). The phylum Proteobacteria was the most abundant in both microbiomes, comprising 39.6% in MASE and 36.4% in SMI (Fig. 1).

Acidobacteria were the second most abundant, with 11.6% in MASE and 24.2% in SMI microbiomes. Actinobacteria were found in higher proportion in MASE soils (19.0%) than in SMI soils (14.2%).

By employing the 16S rDNA gene, a total of 197 genera were identified, 59 of these were found in both types of soils; however, many were only identified as uncultured or unidentified genera. Table 3 shows 37 bacterial genera that were among the most abundant in both type of samples (37% in MASE and 35.85% in SMI), highlighting the genus *Bradyrhizobium*, and uncultured organisms within the family Rhodospirillaceae, whose presence was higher in the MASE soils (6.59%) than in the SMI soils (3.27%).

In terms of fungi, the phylum Ascomycota was the most abundant in both soil samples, representing 26.5% of the total sequences (31.9% in MASE and 21.1% in SMI) (Fig. 2). The phylum Glomeromycota was only identified in the SMI soils with 11.6%, Basidiomycota was identified with 3.2% of the total sequences (0.6% in MASE and 5.8% in SMI), Zygomycota was found in 0.8% of the total sequences (0.5% in MASE and 1.1% in SMI). On the other hand, 63.1% of the fungal sequences in the MASE soils and 10.6% in the SMI soils, were not classified or unidentified, while 49.8%

Table 3 Abundance of different taxonomic levels of bacteria found in MASE and SMI soils

Phylum	Family	Genus	MASE (%)	SMI (%)
Proteobacteria	Rhodospirillaceae	Uncultured	6.59	3.27
Proteobacteria	Bradyrhizobiaceae	*Bradyrhizobium*	3.77	5.55
Gemmatimonadetes	uncultured	Uncultured	3.36	5.31
Proteobacteria	Nitrosomonadaceae	Uncultured	2.53	3.74
Proteobacteria	Comamonadaceae	*Ramlibacter*	2.4	0.23
Actinobacteria	Catelliglobosispora	Uncultured	1.99	0.06
Proteobacteria	DA111	Uncultured	1.8	0.53
Proteobacteria	Sinobacteraceae	*Steroidobacter*	1.56	1.84
Proteobacteria	Sinobacteraceae	Uncultured	1.51	1.4
Proteobacteria	wr0007	Uncultured	1.37	1.78
Proteobacteria	Xanthobacteraceae	Uncultured	1.18	2.16
Proteobacteria	Caulobacteraceae	*Phenylobacterium*	1.1	0.35
Proteobacteria	Xanthobacteraceae	*Pseudolabrys*	0.86	2.51
Proteobacteria	Caulobacteraceae	Uncultured	0.81	0.12
Proteobacteria	Methylobacteriaceae	*Microvirga*	0.62	0.53
Bacteroidetes	Chitinophagaceae	Uncultured	0.57	0.06
Proteobacteria	Erythrobacteraceae	*Altererythrobacter*	0.48	0.61
Actinobacteria	Luedemannella	*Luedemannella*	0.48	0.26
Bacteroidetes	Cytophagaceae	*Flexibacter*	0.38	0.12
Proteobacteria	Xanthomonadaceae	*Lysobacter*	0.35	1.11
Proteobacteria	Rhizobiaceae	*Rhizobium*	0.35	0.12
Proteobacteria	Rhodospirillaceae	*Skermanella*	0.35	0.06
Proteobacteria	Incertae_Sedis	*Rhizomicrobium*	0.32	0.06
Proteobacteria	Sorangiineae	Uncultured	0.3	0.18
Proteobacteria	Sorangiineae	*Sandaracinaceae*	0.27	0.26
Proteobacteria	Hyphomicrobiaceae	*Rhodoplanes*	0.24	0.29
Proteobacteria	KF-JG30-B3	Uncultured	0.22	0.85
Actinobacteria	480-2	Uncultured	0.19	0.03
Proteobacteria	wr0007	Uncultured	0.16	0.2
Proteobacteria	Hyphomicrobiaceae	*Pedomicrobium*	0.13	0.5
Actinobacteria	Blastococcus	Uncultured	0.13	0.29
Proteobacteria	Comamonadaceae	Uncultured	0.13	0.12
Actinobacteria	Arthrobacter	Uncultured	0.11	0.64
Proteobacteria	Sphingomonadaceae	*Sphingomonas*	0.11	0.26
Proteobacteria	Hyphomicrobiaceae	*Hyphomicrobium*	0.11	0.23
Verrucomicrobia	Opitutaceae	*Opitutus*	0.11	0.15
Proteobacteria	Oxalobacteraceae	*Janthinobacterium*	0.05	0.09

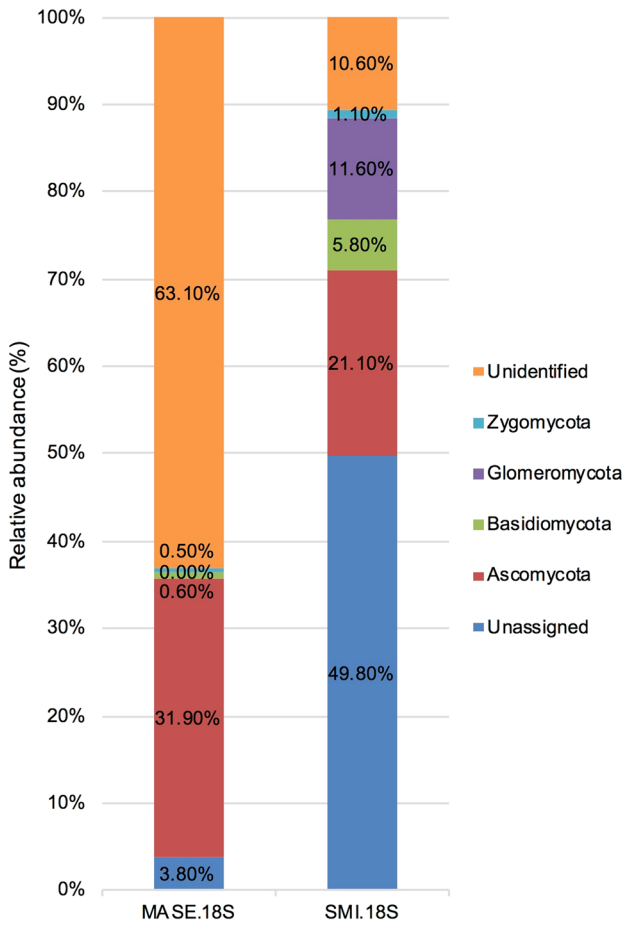

Fig. 2 Relative abundance at Phylum level of fungi from the soil samples. MASE.18S = sample of DNA from agricultural soil analyzed with the 18S rDNA gene; SMI.18S = sample of DNA from mine tailings soil analyzed with the 18S rDNA gene

of the sequences in SMI and 3.8% in MASE, were classified as unassigned sequences (Fig. 2).

Using the 18S rRNA gene sequences, a total of 71 genera were obtained, from which 13 were found in both type of soils, but many represent unidentified genera. A total of 36 fungal genera were found within the most abundant in the soil samples (19.5% in MASE and 27.0% in SMI) (Table 4). The most abundant genera in MASE soils are listed in Table 4, being the genus *Mortierella* (0.49%) within the phylum Zygomycota the only one shared within the SMI microbiome. Other genera in SMI soils are listed in Table 4.

Selection of microorganisms resistant to As

When MASE soil extracts were inoculated on EXSC medium, only 24 bacterial species were able to grow, and none of the fungal species. From these, 18 bacterial isolates were resistant to 5 mM of As (III) (16 isolates) and As (V) (14 isolates). However, their ability to oxidize or reduce As, as shown by the $AgNO_3$ reaction, was negative. On the other hand, a total of 55 bacterial and 9 fungal isolates were able to grow in the EXSC medium from the SMI samples. From the bacterial isolates, 44 were able to grow on 5 mM As (V) and 45 grew on As (III) 5 mM. In this case, 21 bacterial isolates were able to reduce As; however, oxidation of As (III) was not detected. Noteworthy is a colorless halo that was observed in the culture medium supplemented with 5 mM of As (III) in the reaction with $AgNO_3$. This might indicate that the chemical oxidation of As (III) is occurring, in spite of not showing the typical coloration, yellow, for As (III) and reddish-brown for As (V) (Simeonova et al. 2004; Branco et al. 2009).

According to the GenBank database, the As-reducing bacteria belong to the genera *Bacillus* and *Williamsia* (Table 5). Genera *Sphingomonas* and *Nocardioides* showed resistance to 5 mM of As; however, they were unable to reduce it. Similarly, the genus *Lysobacter* was equally resistant to 5 mM of As (III) and (V), but was unable to oxide-reducing. From the nine fungal isolates obtained from the SMI samples, only three showed the ability to reduce As (V), all identified within the genus *Penicillium* (Table 5).

Detection of genes involved in the detoxification and metabolism of As in the microbial cell

From the 21 bacterial isolates showing reduction activity of As, five of them displayed a significant growth in the EXSC medium supplemented with As. These isolates were selected to explore the presence of the five genes related to the As metabolism in bacteria, mentioned above. In spite of finding amplicons with the expected size of the five genes in the five selected isolates, only the *arsB* gene was identified by its sequence. This gene codes for the arsenite transport protein and explains the ability of the five isolates to reduce As (V). All five isolates belong to the genus *Bacillus*, and their identification by their 16S rDNA sequence was: *Bacillus* sp. isolate 2, *B. simplex* isolate 3, *B. simplex* isolate 9, *B. simplex* isolate 47, and *B. megaterium* isolate 49. The isolates 2, 3, and 47 may contain the gene *arrA* which codes for the arsenic reductase, a very important element of the microbial arsenic reduction system with an amplicon of approximately 330 bp. However, their sequences were ambiguous and only indicated that the PCR product was part of the *Bacillus* genome as reported by (Mirza et al. 2017).

Effect of two selected microorganisms on total As in the culture medium

Bacillus simplex isolate 47 was able to decrease the total As of the medium, from a mean of 5.554 mM to a mean of 5.092 mM, which is highly significant ($p = 0.0007$) and indicates a 7.2% decrease of total As in the medium 72 h after

Table 4 Abundance of different taxonomic levels of fungi found in MASE and SMI soils

Phylum	Family	Genus	MASE (%)	SMI (%)
Ascomycota	Nectriaceae	*Fusarium*	3.77	
Ascomycota	Incertae sedis	*Acremonium*	3.51	
Ascomycota	Nectriaceae	*Haematonectria*	2.80	
Ascomycota	Dipodascaceae	Unidentified	1.70	
Ascomycota	Lasiosphaeriaceae	*Cladorrhinum*	1.68	
Ascomycota	Pleosporaceae	*Epicoccum*	1.59	0.04
Ascomycota	Incertae sedis	*Microdochium*	0.76	
Ascomycota	Pleosporaceae	Other	0.60	
Zygomycota	Mortierellaceae	*Mortierella*	0.49	0.47
Ascomycota	Incertae sedis	Other	0.39	
Ascomycota	Incertae sedis	*Stachybotrys*	0.39	
Ascomycota	Orbiliaceae	*Arthrobotrys*	0.36	
Ascomycota	Sordariaceae	Unidentified	0.33	
Ascomycota	Lasiosphaeriaceae	Other	0.26	0.19
Ascomycota	Sporormiaceae	*Westerdykella*	0.21	
Ascomycota	Nectriaceae	Other	0.19	
Basidiomycota	Incertae sedis	*Hannaella*	0.14	
Basidiomycota	Ceratobasidiaceae	*Ceratobasidium*	0.13	
Ascomycota	Lasiosphaeriaceae	Unidentified	0.10	
Ascomycota	Incertae sedis	*Ilyonectria*		8.54
Glomeromycota	Glomeraceae	Unidentified		8.32
Basidiomycota	Inocybaceae	*Neopaxillus*		2.76
Basidiomycota	Ceratobasidiaceae	Unidentified		2.18
Glomeromycota	Acaulosporaceae	*Entrophospora*		1.65
Glomeromycota	Glomeraceae	*Funneliformis*		0.75
Basidiomycota	Tricholomataceae	*Mycenella*		0.49
Glomeromycota	Ambisporaceae	*Ambispora*		0.48
Ascomycota	Trichocomaceae	*Penicillium*		0.21
Ascomycota	Nectriaceae	*Cylindrocarpon*		0.14
Basidiomycota	Filobasidiaceae	*Cryptococcus*		0.13
Ascomycota	Incertae sedis	*Tetracladium*		0.13
Ascomycota	Incertae sedis	*Mycocentrospora*		0.11
Ascomycota	Nectriaceae	*Calonectria*		0.11
Glomeromycota	Ambisporaceae	Other		0.11
Basidiomycota	Ceratobasidiaceae	Other		0.10
Ascomycota	Incertae sedis	*Periconia*	0.08	0.08

being inoculated, with respect to the uninoculated control medium (EXSC + AsIII) (Fig. 3). This isolate also proved to be a reducer of As (V), identified by the yellow coloration in the reaction with $AgNO_3$ (Simeonova et al. 2004). It decreased the total As from a mean of 6.159 mM to a mean of 5.510 mM, which is highly significant ($p = 0.0014$) and indicates a 9.8% decrease of total As in the medium 72 h after being inoculated, with respect to the uninoculated control medium (EXSC + AsV). Isolate 44 (*P. rubens*), showed no decrease of total As in the medium with As (III); however, it decreased the total content of As in the medium, from a mean of 5.805 mM to a mean of 5.144 mM, which is highly significant ($p = 0.0166$) and indicates a 12.1% decrease of total As 144 h after being inoculated, with respect to the control medium (EXSC + AsV) (Fig. 3).

Discussion

Since colonial times, Guanajuato has been economically important to Mexico for two reasons: agriculture and mining industry; and in both economic activities, soil is a preponderant source. Therefore, safety is paramount when handling this natural resource, either for the food industry as well as for the healthy handling of mining sources. The presence of arsenic (As) in Guanajuato's soils has been known since a

Table 5 Identity of As-reducing bacteria and fungus isolates according to their similarity to the NCBI reference sequences

Isolate	% coverage	*E* value	% identity	Closest sequence	Accession number	Identification
2	99	0.0	99.93	HQ317155.1	MN498033	*Bacillus* sp.
3	99	0.0	100.00	KJ831621.1	MN498034	*Bacillus simplex*
9	100	0.0	98.72	MG645295.1	MN498035	*Bacillus simplex* strain LRV34
20	99	0.0	100.00	MG011540.1	MN498036	*Bacillus muralis* strain HlS3200905
47	100	0.0	99.86	MF581431.1	MN498037	*Bacillus simplex* strain Md1-25
49	100	0.0	99.93	MK544829.1	MN498038	*Bacillus megaterium* strain LB11
51	100	0.0	99.66	MK110993.1	MN498039	*Bacillus* sp. strain Whitaker B12
59	99	0.0	99.28	KM374746.1	MN498040	*Bacillus megaterium* strain 1S7
61	89	0.0	84.67	EU073114.1	MN498041	*Williamsia* sp. SY3
44	97	0.0	99.50	LT558870.1	MN493046	*Penicillium rubens*
67	99	0.0	99.02	DQ681334.1	MN493047	*Penicillium granulatum* isolate 732
69	99	0.0	99.65	MK267409.1	MN493048	*Penicillium chrysogenum* isolate E20332

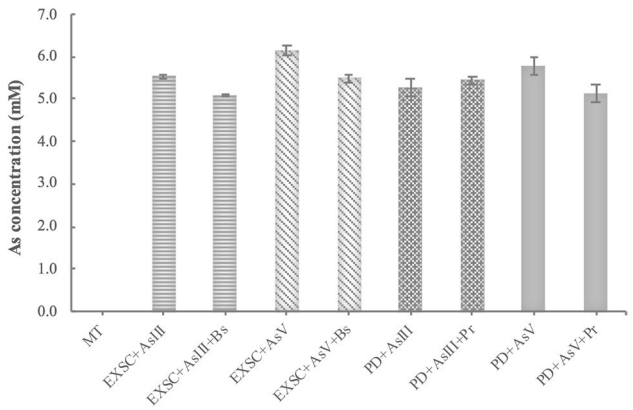

Fig. 3 Quantification of total As in the supernatant of the culture medium, determined by Atomic Absorption Spectrometry. MT = control medium without As and without microorganisms. EXSC + AsIII = medium supplemented with 5 mM of As (III) without microorganisms. EXSC + AsIII + Bs = medium with 5 mM of As (III) and the isolated 47 inoculated. EXSC + AsV = medium supplemented with 5 mM of As (V) without microorganisms. EXSC + AsV + Bs = medium with 5 mM of As (V) and the isolated 47 inoculated. PD + AsIII = medium supplemented with 5 mM of As (III) without microorganisms. PD + AsIII + Pr = medium with 5 mM of As (III) and the isolated 44 inoculated. PD + AsV = medium supplemented with 5 mM of As (V) without microorganisms. PD + AsV + Pr = medium with 5 mM of As (V) and the isolated 44 inoculated

long time (Ramos-Arroyo et al. 2004), but it has never been associated to its microbiome. Resistance of soil microorganisms to toxic elements such as As has made them play a very important role in the mobilization, biotransformation, and bioavailability of different species of As (Cai et al. 2009; Mellado et al. 2011). The SMI soils reported here were more contaminated with As than MASE soils. In MASE soils, the acid pH (6.1) observed could be considered acceptable to achieve good availability of macro and microelements, and no deficiencies of essential nutrients in crops are observed. However, the SMI soils showed a basic pH (8.1), indicating deficiencies in the availability of P, Fe, Mn, Zn, Cu, Co, among others (Brady 1984; Nuñez 1985). Conversely, as it was observed by Ramos-Arroyo et al. (2004), in SMI soils, the basic pH values could be explained for the presence of calcite in the mine tailings, increasing the pH in the soil.

The importance of the great diversity of microorganisms found in soils is highly related to the soil's ability to deal with highly contaminating elements. In previous studies (Campos et al. 2007; Cai et al. 2009), soil microorganisms were able to oxidize or reduce As species in the medium, eliminating (at least partially) its toxicity. Gram-negative bacilli such as *Pseudomonas alcaligenes* and *Wautersia solanacearum*, as well as other bacterial genera such as *Acinetobacter*, *Agrobacterium, Arthrobacter, Comamonas, Rhodococcus, Stenotrophomonas* and *Pseudomonas*, were found to be able to tolerate high concentrations of As (8–20 mM). They also observed that the ability of these bacteria to oxidize As (III) would favor the colonization of other species not tolerant to As, which are important in biogeochemical cycles.

Both soil types (MASE and SMI) studied here showed the presence of Proteobacteria, Acidobacteria, and Actinobacteria, similar to those found previously in Cd-contaminated soils (Feng et al. 2018). In general, Proteobacteria provide some basic functions related to biogeochemical cycles (Feng et al. 2018). As for Acidobacteria, they have been detected in a wide variety of environments. In fact, there are more than 3000 sequences in public databases, shown to be abundant in soils and sediments with the ability to resist extreme conditions (Barns et al. 2007). There is a strong correlation between the abundance of Acidobacteria in soil and its pH, as they are more abundant in soils with pH lower than 6.0, and very uncommon in soils with pH higher than 6.5 (Sait et al. 2006). However, we found abundant Actinobacteria at pH 6.4 for MASE and pH 8.1 for SMI, probably due to the

great versatility of this phylum to adapt to different environments. It is important to notice that, in the case of SMI soils, they contain traces of mine tailing, which were subjected to a geochemical process that changed the original pH of the soil. In spite of the oxidation of sulfides that generated acidity, neutralization and alkalization by carbonates and soil weathering followed (Ramos-Arroyo and Siebe-Grabach 2006), which perhaps gave the Acidobacteria time to adapt to the SMI soils. Perhaps, that is the reason why more Acidobacteria resistant to As were found in this report.

On the other hand, Actinobacteria were found in a higher proportion in MASE soils (19.0%) than in SMI soils (14.2%). Most of these bacteria play an important role in the decomposition of organic matter and they renew the reserves of nutrients in the soil and are fundamental in the formation of humus (Battistuzzi and Hedges 2009). May be that is the reason why Actinobacteria were more abundant in MASE soils as they showed higher organic matter content (8.41%) and TN (0.38%) as compared to SMI soils (7.14 and 0.17%, respectively) (Fig. 1). In fact, Porta et al. (2003) indicate that an optimum C/N ratio with a value close to 10 (MASE C/N ratio was 11.86) suggests a good relationship between mineralization and humification, with higher rates of organic matter degradation.

The genus *Bradyrhizobium* was one of the most abundant in both soil samples, this genus is known for its symbiotic nitrogen fixation in plants (Kaneko et al. 2002). Its presence in the agricultural soil could be related to the presence of crops and its relationship with the improvement of plant nutrition. On the other hand, the presence of the genus *Bradyrhizobium* in the SMI soils may also be related to the native vegetation found in the sampled area, promoting their growth (Corbera-Gorotiza and Nápoles-García 2013). Also, the Family Rhodospirillaceae was found in both soils. The so-called purple non-sulfur bacteria, include a total of 34 genera, and some of them contain several diazotrophic, plant-associated bacteria with growth-promoting potential (Baldani et al. 2014).

Besides, two As-reducers bacterial genera, *Bacillus* and *Williamsia* were identified. The first one is highly diverse and some species act as bio-protectants as well as plant-growth promoters, it is widely distributed and their main habitat is the soil (Orberá Ratón et al. 2005). In general, *Bacillus* species have been known to be good As-resistant bacteria. Since 1998, they were found to resistant As and can reduce it (Blum et al. 1998; Suresh et al. 2004) and absorb both As (III) and As (V) (Yang et al. 2012). As for *Williamsia*, it usually participates in the biodegradation process and decomposition of contaminated industrial soils (Keikha 2018). *Williamsia* is a genus that has been isolated from As contaminated soils and classified as novel arsenite-resistant bacteria (Cai et al. 2009; Šimonovičová et al. 2016; Wu et al. 2018), but no extensive studies have been done which leaves a wide research area to explore. We found the above-mentioned bacterial genera in both types of soils, similar to a previous report (Campos et al. 2007); however, some of these isolates may represent new bacterial strains resistant to As. Also, the search for newer potent As-resistant bacteria in a specific ecological niche of contaminated soil is always relevant (Srivastava et al. 2013; Ghosh et al. 2018).

In general, fungi are abundant microorganisms in soil. They represent a large proportion of the soil's microbial biomass. The main groups of fungi found in the studied soils are Zygomycota, Glomeromycota, Ascomycota, and Basidiomycota. Within the Phylum Ascomycota the most frequently found genera in soil are *Penicillium* and *Aspergillus* (Gondim-Porto 2013). Fungi within the genus *Penicillium* are very diverse and play an important role in the decomposition of organic matter and the production of enzymes (Visagie et al. 2014). We report three species of this genus in the presence of As, especially *P. rubens* which grew in medium supplemented with As (III) at concentrations higher than 10 mM, contrary to the bacteria, which develop best in medium supplemented with As (V), and at lower concentration (Mellado et al. 2011). The Phylum Glomeromycota found only in the SMI soils form arbuscular mycorrhizae (Schüßler et al. 2001). Its presence in SMI soils may be related to the plants growing in the sampled area.

The mobility, availability, and speciation of As are affected by the microbial metabolism that participates in the biogeochemical cycle of this element. Among the microbial activities, some strains have been found to be able of oxidizing arsenite or reducing arsenate. The *ars* operon constitutes the most important and studied scheme of arsenic tolerance in microorganisms. Some genes involved are the *arsC* gene, for the arsenate reductase capable of transforming As (V) to As (III), present in bacteria that have been characterized in terms of their resistance to As (Jackson et al. 2005). Cai et al. (2009) found genes involved in the oxidation of As (III): the *aoxB* gene that codes for the major subunit of the arsenite oxidase, as well as the genes that code for different arsenite transporting proteins in the microbial cell (*arsB, ACR3 (1) and ACR3 (2)*), found in bacteria isolated from soil highly contaminated with As (20 mM).We successfully amplified the *arsB* gene in the bacterial isolates with the ability to reduce As (V), all within the genus *Bacillus* (*Bacillus* sp., *B. simplex* and *B. megaterium*). This gene confers the bacteria the ability to be more resistant to As, catalyzing the extrusion of As (III) outside the cell (Mukhopadhyay and Rosen 2002). The *B. simplex* isolate 47 was able to decrease the total As by 7.2%; however, is considered a low proportion as another *Bacillus* strain, was able to remove 76.0% of As (V) at a concentration of 6 mM and 56.0% was reduced to As (III) (Tripti et al. 2014).

In soils contaminated with As, it is common that microorganisms develop mechanisms to incorporate the metalloid into their metabolism. The soils studied here provide important information on native species of microorganisms resistant to As. On the other hand, a higher content in organic matter and moisture in MASE, in contrast with SMI soils, may contribute to its richest microbiome, which may explain that a greater diversity of bacteria was found in these soils, as compared to the mining soils.

In the SMI soils (highly contaminated by As), a greater number of As-resistant microorganisms were found, with the ability to transform the chemicals species of As (reduction of As (V) to As (III)), as compared to microorganisms isolated from MASE soils. Although the phylum Firmicutes was found in a very low proportion in the SMI soil, they were able to reduce As (V) to As (III). On the other hand, the fungus *Penicillium* showed the greatest ability to reduce the As (V) to As (III) and a strong resistance to high concentrations of As (III) (> 10 mM).

It is important to notice that the origin of As in both types of soils may be different. Weathering of different parent material (rocks) in the catchment area of mines commonly contributes to the As content of SMI soils. Conversely, MASE soils come from weathering of volcanic rocks, rich on Na, Fe, Mg, and Fe–Ti oxides (Nieto-Samaniego et al. 2012). Therefore, As found in MASE soils is mostly related to irrigation with As-rich groundwater from deep boreholes (Zanor et al. 2019). The origin of As in both types of soils may explain the low ability of bacteria to process it on MASE soils, as well as the high ability to process As by fungi in SMI soils. In other words, the microbiome in each type of soil is the result of the ability of different microbiota to survive under different conditions.

The results obtained in this work can contribute to a better understanding of the microbiomes of soils contaminated with As. Furthermore, a fungus showed the highest ability to process As, rather than a bacterium, which are most common microorganisms to degrade As in nature. This observation is important as fungi uses a different metabolic pathway to deal with As, and also to look forward to find microorganisms with biotechnological potential in the restoration and bioremediation of As-contaminated soils, in the future.

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Authors' contributions MELP and MCDRC conceived the study. MELP, GAZ, and ASR performed the research. MELP and MCDRC analyzed data and wrote MS, JEI interpreted and analyzed the data, structured and wrote the MS. All authors read and approved the manuscript.

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Compliance with ethical standards

Conflict of interest Authors declare that they have no conflict of interest.

Ethical approval This work does not involve any study with human participants or animals.

Consent to participate All authors consent to participate.

Consent for publication All authors consent for publication of this paper.

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