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Unique diversity and functions of the arsenic-methylating microorganisms from the tailings of Shimen Realgar Mine

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Abstract

Microbial arsenic (As) methylation plays important roles in the As biogeochemical cycle. However, little is known about the diversity and functions of As-methylating microorganisms from the tailings of a Realgar Mine, which is characterized as containing extremely high concentrations of As. To address this issue, we collected five samples (T1–T5) from the tailings of Shimen Realgar Mine. Microcosm assays without addition of exogenous As and carbon indicated that all the five samples possess significant As-methylating activities, producing 0.8–5.7 μg/L $DMAs^V$, and 1.1–10.7 μg/L $MMAs^V$ with an exception of T3, from which $MMAs^V$ was not detectable after 14.0 days of incubation. In comparison, addition of 20.0 mM lactate to the microcosms significantly enhanced the activities of these samples; the produced $DMAs^V$ and $MMAs^V$ are 8.0–39.7 μg/L and 5.8–38.3 μg/L, respectively. The biogenic $DMAs^V$ shows significant positive correlations with the Fe concentrations and negative correlations with the total nitrogen concentrations in the environment. A total of 63 different *arsM* genes were identified from the five samples, which code for new or new-type ArsM proteins, suggesting that a unique diversity of As-methylating microbes are present in the environment. The microbial community structures of the samples were significantly shaped by the environmental total organic carbon, total As contents and NO_3^- contents. These data help to better understand the microorganisms-catalyzed As methylation occurred in the environment with extremely high contents of As.

Keywords Arsenic methylation · ArsM · Microbially As methylation · Shimen Realgar Mine · Arsenic contaminated soils

Introduction

Arsenic (As) is a natural component of the earth′s crust and is widely distributed in water, air and soils. The average content of As in the crust is approximately 2.0 mg/kg (Oremland and Stolz 2003; Kulp 2014; Wang et al. 2017). As is found in both organic and inorganic forms. Inorganic As compounds, such as arsenite [As(III)] and arsenate [As (V)], are highly toxic to organisms, while organic As compounds are generally less harmful to health (Oremland and Stolz 2003; Zhu et al. 2014, 2017; Maguffin et al. 2015). Exposure to inorganic As by drinking contaminated water or eating As-containing foods pose a significant health risk for people around the world. Long-term exposure to inorganic As can lead to cardiovascular disease, lung diseases, diabetes, various cancers, neurotoxicity, immune alterations, and reproductive disorders (Zhu et al. 2014; Singh et al. 2015). It is now recognized that at least 200 million people in more than 70 countries worldwide have been utilizing groundwater containing As at levels above the WHO provisional guideline value of 10 μg/L (Nordstrom 2002; Bhattacharya et al. 2007; Schaefer et al. 2016). Therefore, it is important and urgent to fully understand the biogeochemical cycles of As, and thus design environment-friendly strategy to remediate the As-contaminated soils and groundwater.

Increasing investigations suggest that microorganisms play key roles in the global geochemical cycles of As. They catalyze As oxidization, reduction and methylation (Li et al. 2016; Chen et al. 2017; Zeng et al. 2018b). A(III)-oxidizing bacteria (AOB) can convert As(III) into As(V) under either aerobic or anaerobic conditions. Under aerobic conditions, AOB can convert As(III) into As(V) by using oxygen as the sole electron acceptor (Zeng et al. 2016, 2018a; Bagade et al. 2016), while under anaerobic conditions, AOB can oxidize As(III) by using nitrate, nitrite, selenate or chlorate as the

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sole electron acceptor (Rhine et al. 2008; Fisher and Hollibaugh 2008; Sun et al. 2011; Zhang et al. 2015a). Typical As(V)-reducing microbes are dissimilatory As(V)-respiring prokaryotes (DARPs). DARPs are able to reduce As(V) into As(III) by using organic materials, such as lactate, pyruvate, citrate, glucose, succinate, glycerin, D-fructose, L-malate, Propionate, acetate and aromatic compounds, or inorganic chemicals, such as sulfide and hydrogen, as the sole electron donor (Lear et al. 2007; Song et al. 2009; Ohtsuka et al. 2013; Osborne Kulp et al. 2015; Shi et al. 2018). DARPs are considered to be the major drive for the mobilization and release of As from soils and minerals into aqueous phase (Kudo et al. 2013; Osborne Kulp et al. 2015; Chen et al. 2017; Wang et al. 2017). Both AOB and DARPs are widely distributed in the As-contaminated soils, sediments, water, mines and tailings. Some cultivable AOB and DARP strains were isolated from diverse environment for the investigations of the molecular and physiochemical features of the As metabolizing bacteria, and their interactions with As-bearing minerals (Zeng et al. 2016; Wang et al. 2017; Chen et al. 2017; Yang et al. 2017).

Recently, As-methylating microorganisms (AMMs) are catching people's attentions because they can lead to volatilization of environmental As (Wang et al. 2014, 2015; Xue et al. 2017). Microbial methylation detoxifies As by converting As(III) into methylated As species, such as $MMAs^{V}$, $DMAs^{V}$, TMAO, and volatile $TMAs^{III}$ (Qin et al. 2006, 2009; Slyemi and Bonnefoy 2012; Chen et al. 2014; Zhu et al. 2014). This reaction is catalyzed by arsenic S-adenosylmethionine methyltransferase (ArsM) (Ajees et al. 2012). The *arsM* gene is thus used as a molecular marker for the detection of the existence and diversity of AMMs from the environment (Jia et al. 2013). AMMs were detected in diverse environment, including As-contaminated soils, sediments, groundwater, wetlands, and wastewater (Kuramata et al. 2015; Zhang et al. 2015b; Huang et al. 2016; Wang et al. 2016; Zeng et al. 2018b). Some cultivable AMM strains were also isolated from the environment for the investigations of their activities, taxonomy or ArsM features (Kuramata et al. 2015; Zhang et al. 2015b; Huang et al. 2016, 2017). Microbial As methylation was applied for the bioremediation of As contaminated environment by converting As(III) into volatile methylated species with less toxicity (Maguffin et al. 2015; Srivastava et al. 2011; Mestrot et al. 2013). To enhance the bacterial As methylation activities for bioremediation, recombinant *arsM* genes were transferred into a bacterial host for achieving higher expression levels of ArsM proteins (Qin et al. 2006; Ye et al. 2014; Wang et al. 2014, 2015).

The Shimen Realgar Mine had been the largest one in Asia for more than 1500 years. However, it was closed since 1978 due to severe environmental contaminations caused by smelting of arsenic. It was found that there are four types of secondary minerals present in the tailings of this mine: As oxides, sulfur-bearing As(V), As–gypsum and As–Fe minerals (Zhu et al. 2015). Our previous investigations revealed that there are unique diversity of As(III)-oxidizing bacteria in the tailings, which possess high As (III)-oxidizing activities, and are either autotrophic or heterotrophic (Zeng et al. 2016). We also found that there are DARPs in the tailings, which are able to catalyze the mobilization and release of As from the mineral phase into pore water (unpublished data). This study aimed to explore the diversity and activities of AMMs in the tailings. We also examined the relationship between the AMM activities and the environmental factors. This work highlights the unique diversity and functional feature of AMMs in the environment with extremely high concentrations of As.

Materials and methods

Sampling

Five different samples with high concentrations of As were collected from the depth of approximately 50 cm of the tailings in Shimen Realgar Mine affiliated to the Baiyun town of Shimen county, Changde city of Hunan province, China. The site was located at N29°33′55.39″, E111°01′45.06″ (Fig. 1). The samples were placed in sterile 50-mL tubes, and kept on ice. All samples were delivered into the lab immediately.

Chemical analyses

Total As in the tailing was solubilized with aqua regia and examined using the Atomic Fluorescence Spectrometry (AFS-9600, Haiguang, China) as described previously (Wang et al. 2017). Soluble As species, including As(III), As(V), $DMAs^{V}$ and $MMAs^{V}$, were measured by high-performance liquid chromatography coupled with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) (LC-20A, Shimadzu, Japan; Aglilent 7700, Aglilent, USA). Concentrations of ammonium were measured with Nessler assay as described previously (Kim et al. 2006). Concentrations of nitrate were determined with thymol reagent (Broderick et al. 2005). Sulfate and phosphate were determined using ion chromatography (IC-761, Metrohm, Switzerland). The pH value was determined by a pH analyzer (PHSJ-4F, REX, China). The contents of total organic carbon (TOC) was determined using a TOC analyzer (liquiTOC, Elementar, Germany). Total nitrogen (TN) was measured with a TN analyzer (TNP-4110, Shimadzu, Japan).

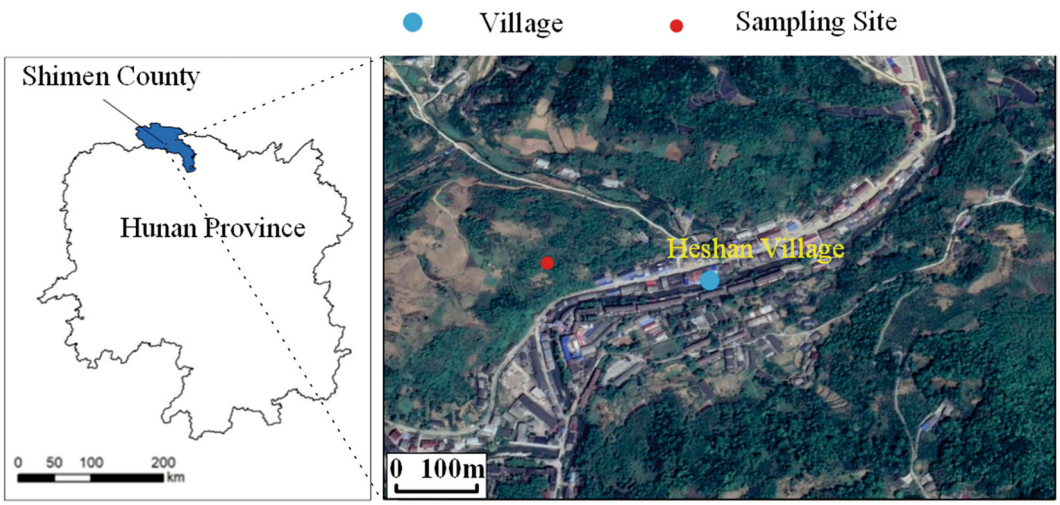

Fig. 1 The geographical map of the sampling site

Table 1 PCR primers used for this study

Target genes	Primer sequences	References
arsM		Jia et al. 2013
arsMF1	TCYCTCGGCTGCGGCAAYCCVAC	
arsMF2	GTGCTCGAYCTSGGCWCCGGC	
arsMF3	GGCATCGACGTGCTKCTBTCSGC	
arsMR1	AGGTTGATGACRCAGTTWGAGAT	
arsMR2	CGWCCGCCWGGCTTWAGYACCCG	
arsMR3	GCGCCGGCRAWGCAGCCWACCCA	
16S rRNA gene V3-V4		Wu et al. 2016
520F	AYTGGGYDTAAAGNA	
802R	TACNVGGGTATCTAATCC	
T vector		Wang et al. 2017
M13-47	CGCCAGGGTTTTCCCAGTCACGAC	
RV-M	GAGCGGATAACAATTTCACACAGG	

Detection of microbial As-methylating activity of the samples

Microcosm assay was used to examine the As-methylating activity of the microbial community from each sample as described previously (Zeng et al. 2018b). Active microcosms were prepared in triplicate by mixing approximately 8.0 g of samples with 20.0 mL sterile water containing 20.0 mM lactate or without lactate in 50-mL tubes. The mixtures autoclaved at 120 °C for 20 mins were served as controls. All tubes were incubated at 30 °C without shaking. After 80.0 days, approximately 1.0 mL of slurries was removed from the tubes for measuring the concentration of $DMAs^V$ and $MMAs^V$ by HPLC-ICP-MS.

Cloning and analysis of microbial arsM genes from the total genomic DNA of samples

The *arsM* gene-specific primers were listed in Table 1. Total DNA from a sample was prepared using Quick Extract-Bacterial DNA Extraction Kit (Epicentre, Madison, Wisconsin, USA). The *arsM* genes were each amplified with PCR reactions. PCR products were each separated with a DNA agarose gel electrophoresis. DNA band was cut out, and DNA was extracted using TaKaRa Gel Extraction kit (TaKaRa Biotechnology, Japan). Purified DNA was cloned into T-vector for high-throughput sequencing. The obtained DNA sequences were analyzed with BLAST server and MEGA 6.0 software package as described previously (Zeng et al. 2018b; Shi et al. 2018).

Analysis of the microbial community structures

Total genomic DNA was extracted from the tailing samples using Soil DNA Extraction Kit (Omega, USA). The microbial 16S rRNA genes of the microbial communities from the samples were each amplified, sequenced, and analyzed as described previously (Zeng et al. 2016).

Correlation analysis

Spearman's Rank Correlation was used to determine the correlations between As-methylating activities and the environmental factors, such as NH_4^+, SO_4^{2-}, NO_3^-, TOC, TN, Fe, total As, and soluble As (Zeng et al. 2018b). Correlations were considered to be statistically significant at a 95% confidence level ($P < 0.06$).

Results

Geochemical characterization of the samples

We collected five samples (named as T1–T5) from a representative site of the tailings of Shimen Realgar Mine. The site was located at N29°33′55.39″, E111°01′45.06″. Geochemical analysis indicated that the samples T1, T2, T3, T4 and T5 had extremely high contents of As, containing 1202.3, 277.6, 1908.3, 31990.6, and 96.7 mg/kg of total As, and 186.60, 0.10, 601.19, 545.79, and 29.29 mg/kg of soluble As, respectively (Table 2). The samples also contained relative high contents of SO_4^{2-} (varied from 58.00 to 328.84 mg/kg), and TOC (from 20.13 to 41.73 mg/kg), and relative low contents of NO_3^- (from 0.10 to 1.61 mg/kg), PO_4^{3-} (from 0.65 to 10.48 mg/kg), TN (from 0.23 to 0.79 mg/kg), and NH_4^+ (from 0.066 to 0.158 mg/kg). Therefore, these tailing samples were characterized as containing extremely high contents of As. The chemical substances in the samples provided basic nutrition for the growth of environmental microorganisms.

Table 2 Geochemical parameters of the five tailing samples

Parameters	Sediment samples				
	T1	T2	T3	T4	T5
pH	6.72	6.73	6.70	6.75	7.09
Total As (mg/kg)	1202.25	277.56	1908.28	31990.61	96.74
soluble As (mg/kg)	186.60	0.10	601.19	545.79	29.29
TOC (mg/g)	20.88	26.51	27.48	41.73	20.13
NH_4^+ (mg/g)	0.081	0.127	0.158	0.076	0.066
NO_3^- (mg/g)	0.36	1.32	0.50	0.10	1.61
SO_4^{2-} (mg/kg)	131.21	149.12	328.84	58.00	51.84
PO_4^{3-} (mg/g)	0.85	0.65	1.20	0.65	10.48
TN (mg/g)	0.52	0.52	0.79	0.23	0.36
Fe (mg/g)	0.02	0.09	0.05	0.01	0.01

Arsenite-methylating activities of the microbial communities from the tailing samples

Microcosm assay was utilized to determine the As-methylating activities of the microbial communities from the five tailing samples in the presence or absence of exogenous carbon. As shown in Fig. 2, in the absence of any exogenous substances, after 80.0 days of incubations, approximately 0.8, 13.3, 2.3, 2.2, and 5.7 μg/L $DMAs^V$, and 1.1, 7.9, 10.7, 0, and 8.4 μg/L $MMAs^V$ were released from the microcosms of T1, T2, T3, T4, and T5, respectively (Fig. 2). In contrast, in the autoclaved slurries, no detectable methylated As species were observed. This suggests that under natural environmental conditions, the microbial communities in all the five tailing samples possess significant As-methylating activities.

Considering that the contents of TOC in the tailings are very limited, we examined the microbial As-methylating activities of the tailing samples in the presence of exogenous organic carbon. The microcosms were amended with 20.0 mM lactate as the carbon source. After 80.0 days of incubations, approximately 39.7, 13.8, 9.2, 8.0, and 13.7 μg/L $DMAs^V$ and 38.3, 37.4, 5.8, 21.0, and 22.1 μg/L $MMAs^V$ were detected in the microcosms of T1, T2, T3, T4, and T5, respectively. No significant amount of methylated As species were detected from the autoclaved slurries prepared from the five samples. This suggests that exogenous organic carbon inputs markedly increased the As-methylating activities of the microbial communities from the tailings.

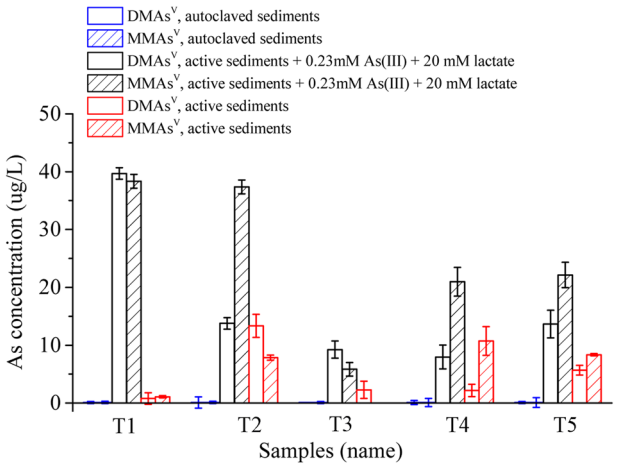

Fig. 2 Arsenic methylation activities of the microbial communities from the five samples collected from the tailings of Shimen Realgar Mine

Unique diversity of ArsM proteins from the microbial communities of the tailings

To understand the diversity of the As-methylating microbes in the tailings, we amplified, cloned, sequenced and analyzed the *arsM* genes from the microbial communities of the five tailing samples. After excluding repetitive sequences, we identified 63 new or new-type microbial ArsM proteins from the microbial communities of the five samples, among which 11, 11, 8, 11, and 22 ArsMs were obtained from the samples T1, T2, T3, T4, and T5, respectively (Fig. 3). Comparison of these ArsM protein sequences showed that they share 55.61–100% sequence identities to each other. Each of the obtained ArsM sequences was used as a query to BLAST against the GenBank database. We found that these new ArsMs share 45.83–92.41% sequences identities with other known ArsM proteins from bacteria and archaea. A phylogenetic tree was constructed based on the alignment of the ArsMs from this study and their closely related homologs from the GenBank database. An ArsM sequence from archaea was chosen as the outgroup (Fig. 3).

If a group of ArsMs form an independent cluster in the phylogenetic tree, and they share less than 60% maximal homology with other known ArsM proteins, we classified them as a new family of ArsMs (Zeng et al. 2018a, b). Based on this criteria, we identified four new families of ArsM proteins from the five samples: family 1 (E-13), family 2 (C-18, C-7, C-2, C-13, C-4, E-9, E-23, E-18, B-1, B-2, B-9, B-14), family 3 (A-1, A-2, A-4, A-6, A-7, A-8, A-11, C-6, E-3, E-7, E-8, E-11, E-20, E-21 and E-22), family 4 (E-17), and family 5 (D-1, D-3, D-7, D-10, D-11 and D-12) (Fig. 3).

It is interesting to see that the ArsMs D-2, D-4, D-5, D-6, E-24, E-10 and E-13 are closely associated with the known ArsM proteins from archaea *Thaumarchaeota* and *Euryarchaeota*; this suggests that the seven new ArsMs from the tailings are likely to come from archaea. Other ArsM proteins obtained from the tailings are closely associated with the known ArsMs from bacterial phyla, such as *Acidobacteria*, *α-Proteobacteria*, or *Nitrospirae* (Fig. 3).

These findings highlight the unique diversity of the As-methylating microorganisms from the tailings of Shimen Realgar Mine.

Correlation between microbial As-methylating activities and environmental factors

We analyzed the correlation between the microbial As-methylating activities and the geochemical parameters of the tailing samples using the Spearman's Rank Correlation method. The results showed that the concentrations of biogenic $DMAs^V$ are positively correlated with those of Fe ($r = 0.90488$; $p = 0.03471$); the concentrations of biogenic $MMAs^V$ show negative correlations with those of TN ($r = -0.86078$; $p = 0.06104$) (Fig. 4a, b), and slight positive correlations with those of Fe ($r = 0.8111$; $p = 0.0957$) and NH_4^+ ($r = 0.80785$; $p = 0.09815$).

In comparison, the concentrations of biogenic $DMAs^V$ show no significant correlations with other geochemical parameters, such as TOC ($r = -0.55535$; $p = 0.33115$), TN ($r = 0.09521$; $p = 0.87895$), SO_4^{2-} ($r = -0.11206$; $p = 0.85761$), and PO_4^{2-} ($r = -0.13839$; $p = 0.82436$); the concentrations of the biogenic $MMAs^V$ show no significant correlations with TOC ($r = -0.29243$; $p = 0.63304$), PO_4^{2-} ($r = -0.50423$; $p = 0.38634$), and NO_3^- ($r = 0.19425$; $p = 0.75424$).

Microbial community structure of the tailing samples

In order to figure out what environmental factors can indirectly affect the microbial As-methylating activities via shaping the microbial community structures from the tailings, we sequenced and analyzed the 16S rRNA genes from the total genomic DNA of the five samples. Using Illumina Miseq high throughput sequencing technique, we obtained a total of 273,690 high-quality 16S rRNA gene sequences from the five samples, among which 71,340, 32,193, 38,152, 73,300, and 58,705 sequences were obtained from the samples T1 T2, T3, T4, and T5, respectively. Rarefaction analysis indicated that these sequences cover 99.7%, 99.5%, 99.5%, 99.9%, and 99.6% of the total microbial diversity of the tailing samples T1, T2, T3, T4, and T5, respectively (Fig. 5b).

As shown in Fig. 5a, we identified 42 phyla of bacteria from the five tailing samples. The dominant phyla in average include *Proteobacteria* (22.45% of the total microbial communities), *Chloroflexi* (18.45%), *Acidobacteria* (18.08%), *Nitrospirae* (10.01%), GAL 15 (7.23%), *Bacteroidetes* (6.56%), *Gemmatimonadetes* (4.11), *Actinobacteria* (2.27%), unclassified_k_norank (2.27%), *Latescibacteria* (1.67%), *Planctomycetes* (1.45%), *Verrucomicrobia* (1.41%), and other less dominant bacteria (4.05%). The structure of the microbial communities from this study significantly differs from that obtained from our previous investigations on the tailings of Shimen Realgar Mine (Zeng et al. 2016); it also dramatically differs from the microbial community structures of the As-contaminated soils and sediments from Jianghan Plain (Chen et al. 2017; Shi et al. 2018).

Pairwise comparison using ANOSIM indicated that there are significant differences among the microbial community compositions of the five samples. Based on the Bray-Curtis dissimilarity analysis using UPGMA, we found that the five samples can be grouped into four groups: I (T4), II (T1 and

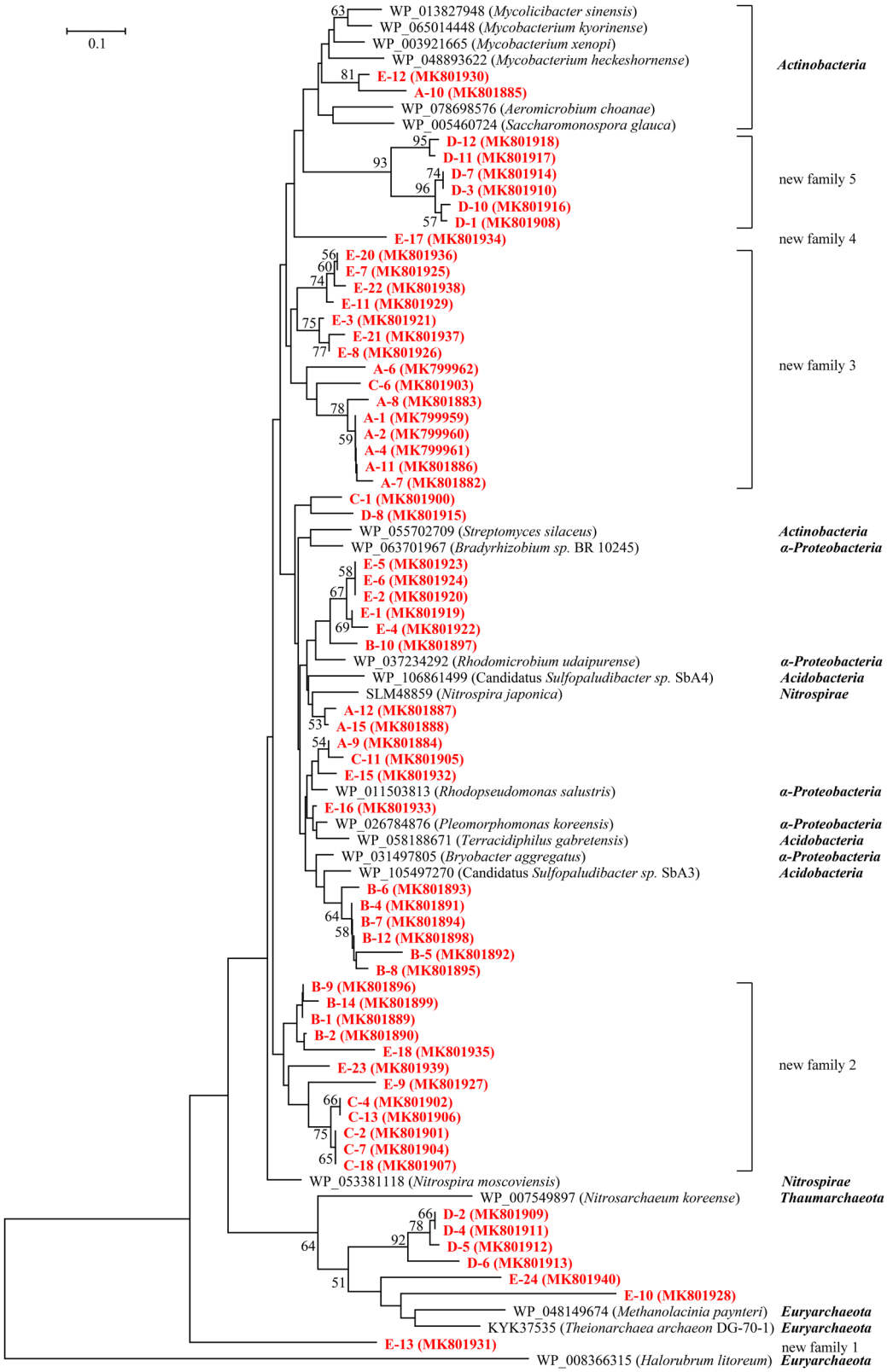

Fig. 3 Correlations of the methylated As species and geochemical parameters

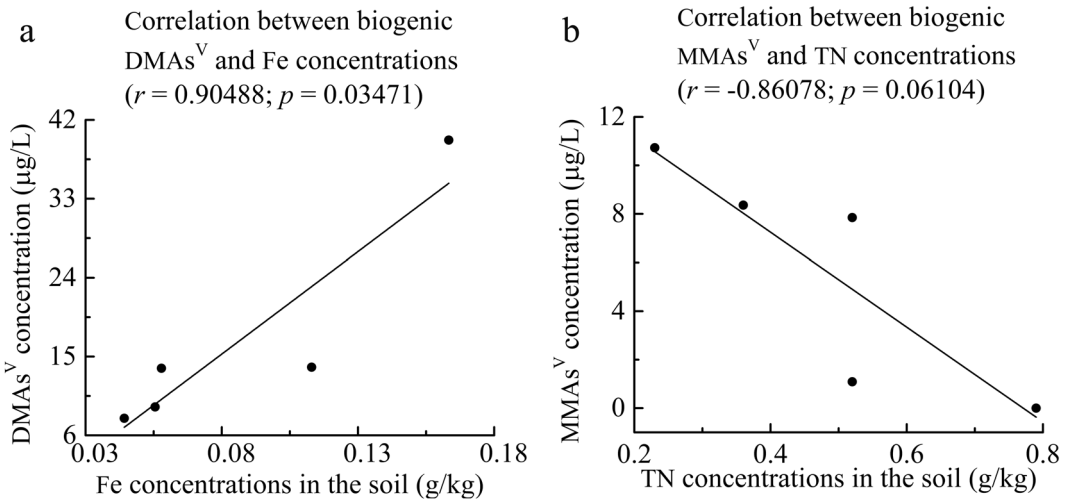

Fig. 4 Phylogenetic analysis of the ArsM proteins identified from microbial communities of the tailing samples from Shimen Realgar Mine

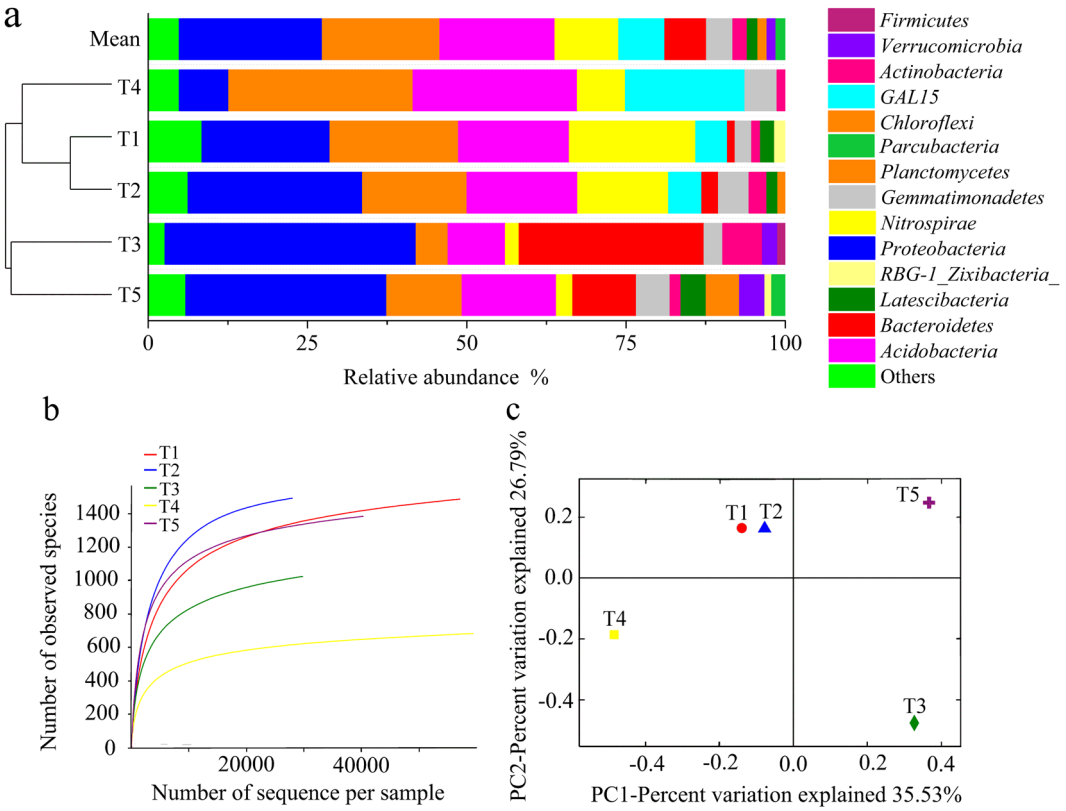

Fig. 5 Compositions of the microbial communities from the tailings of Shimen Realgar Mine. **a** Relative abundances of the dominant phyla from the microbial communities of the tailing samples and UPGMA analysis for the microbial communities. **b** Rarefaction curves of the microbial communities from the tailing samples. **c** Principal-coordinate analysis (PcoA) of the microbial communities from the tailing samples

T2), III (T3) and IV (T5) (Fig. 5a). PCoA analysis also indicated that the five samples were divided into four distinct group (Fig. 5c).

We further analyzed the correlations between the microbial community compositions with the geochemical parameters. We found that the diversity indices of the microbial communities show significant negative correlations with the TOC contents in the samples (TOC and ACE, $r = -0.90867$ and $p = 0.03268$; TOC and Chao, $r = -0.91335$ and $p = 0.03022$; TOC and Shannon, $r =$

−0.90627 and $p = 0.03396$) (Fig. 6a–c). The Shannon diversity was positively correlated with the contents of NO_3^- (Shannon and NO_3^-, $r = 0.87252$ and $p = 0.05358$) (Fig. 6d). The microbial diversity also shows significant negative correlations with the total As contents (TA) (TA and ACE, $r = -0.87683$ and $p = 0.05092$; TA and Chao, $r = -0.87223$ and $p = 0.05376$; TA and Shannon, $r = -0.90845$ and $p = 0.03279$) (Fig. 6e–g). This suggests that the environmental TOC, total As and NO_3^- are the three important environmental factors that indirectly affect the microbial As-methylating activities by shaping the microbial community structures in the tailings of Shimen Realgar Mine.

The other geochemical parameters show no significant correlation with the microbial community diversity of the five tailing samples.

Discussion

The Shimen Realgar Mine had produced pure realgar for hundreds of years; this led to mountains of tailings around the mining areas. In the previous investigations, we found that there are unique diversity of As(III)-oxidizing bacteria in the tailings (Zeng et al. 2016). Using a population of As (III)-oxidizing bacteria from the tailings, we constructed an effective bioreactor, of which As(III)-oxidizing efficiency is at least 6.5–19.0 times higher than other described microbial bioreactors (Li et al. 2016). We also isolated some DARP strains from the soils near the mining site, and found that they can catalyze the reduction, mobilization and release of As from the solids into aqueous phase (Zhu et al. 2019; Wang et al. 2017; Chen et al. 2017). In this study, we focused on exploration of the existence, diversity and activities of As-methylating microorganisms from the tailings.

Generally, the As-contaminated soils and sediments contain 18.89–42.11 mg/kg of total As, and 0.03-0.79 mg/kg of soluble As (Zeng et al. 2016, 2018b; Wang et al. 2017). In comparison, we found that the tailing samples from the Shimen Realgar Mine contain an average of 7095.09 mg/kg of total As, and 272.59 mg/kg of soluble As. Therefore, the tailings provide an ideal condition for the growth of microorganisms that are resistant to extremely high contents of As.

Previous investigations focused on the descriptions of AMMs from the As-contaminated soils, sediments, groundwater, and manure, where As concentrations are relatively very low (Cai et al. 2013; Reid et al. 2017; Zhai et al. 2017; Zhang et al. 2017). In this study, we found that AMMs also exist in the environment with extremely high contents of As. We identified 63 new or new-type ArsM proteins from the tailings. These data highlight unique diversity of As-methylating microorganisms present in the tailings of Shimen Realgar Mine.

We also examined the As-methylating activities of the microbial communities from the tailings using laboratory microcosm assays in the presence or absence of exogenous carbon source. We found that the microbial communities from all the tailings can convert As into $DMAs^V$ and $MMAs^V$, with the exception of the sample T3, which only produced $DMAs^V$. Addition of exogenous carbon significantly increased the microbial As-methylating activities of all the samples; this suggests that although a lot of AMMs exist in the tailings, the microbial As methylation efficiency under natural conditions is very slow, and inputs of external carbon can stimulate the As-methylating reactions. However, the As methylation shows no significant correlations with the TOC contents in the tailings. This unexpected observation may be attributed that the carbon may dominantly stimulate the growth of non-As-methylating microorganisms that may competitively inhibit the growth of AMMs. It is interesting to see that the concentrations of biogenic $DMAs^V$ are positively correlated with those of Fe, whereas the concentrations of biogenic $MMAs^V$ are negatively correlated with those of the total nitrogen. In comparison, for the AMMs from As-contaminated soils and sediments of Jianghan Plain, the biogenic $DMAs^V/MMAs^V$ shows no significant correlations with either Fe, or total nitrogen (Zeng et al. 2018b). This suggests that the activities of AMMs from different habitats are differentially regulated by the environmental factors.

Arsenic is the substrate of ArsM enzyme in the methylation reactions. However, it is unexpected that the activities of AMMs show no significant correlations with either total As, or soluble As in the environment. A comparative analysis also indicated that the average As-methylating activities of the tailing samples from this study are comparable to those of the soils and sediments from Jianghan Plain, although the As contents in the tailings are much higher than those in the soils and sediments (Zeng et al. 2018b). This seems to imply that the content of As is not a key factor affecting the microbial As-methylating activity.

It is noteworthy that the dominant methylated species by AMMs from the As-contaminated sediments of Jianghan Plain is $DMAs^V$ (Zeng et al. 2018b); however, neither $DMAs^V$ nor $MMAs^V$ produced by the AMMs from this study are significantly dominant. The mechanism leading to this difference remains to be elucidated.

Some environmental factors may indirectly affect the activities of AMMs through shaping the structures of microbial communities in the tailings. To verify this hypothesis, we analyzed compositions of the microbial communities from the five samples. We found that TOC and total As are the key factors that significantly affect the structures of the microbial communities from the tailings.

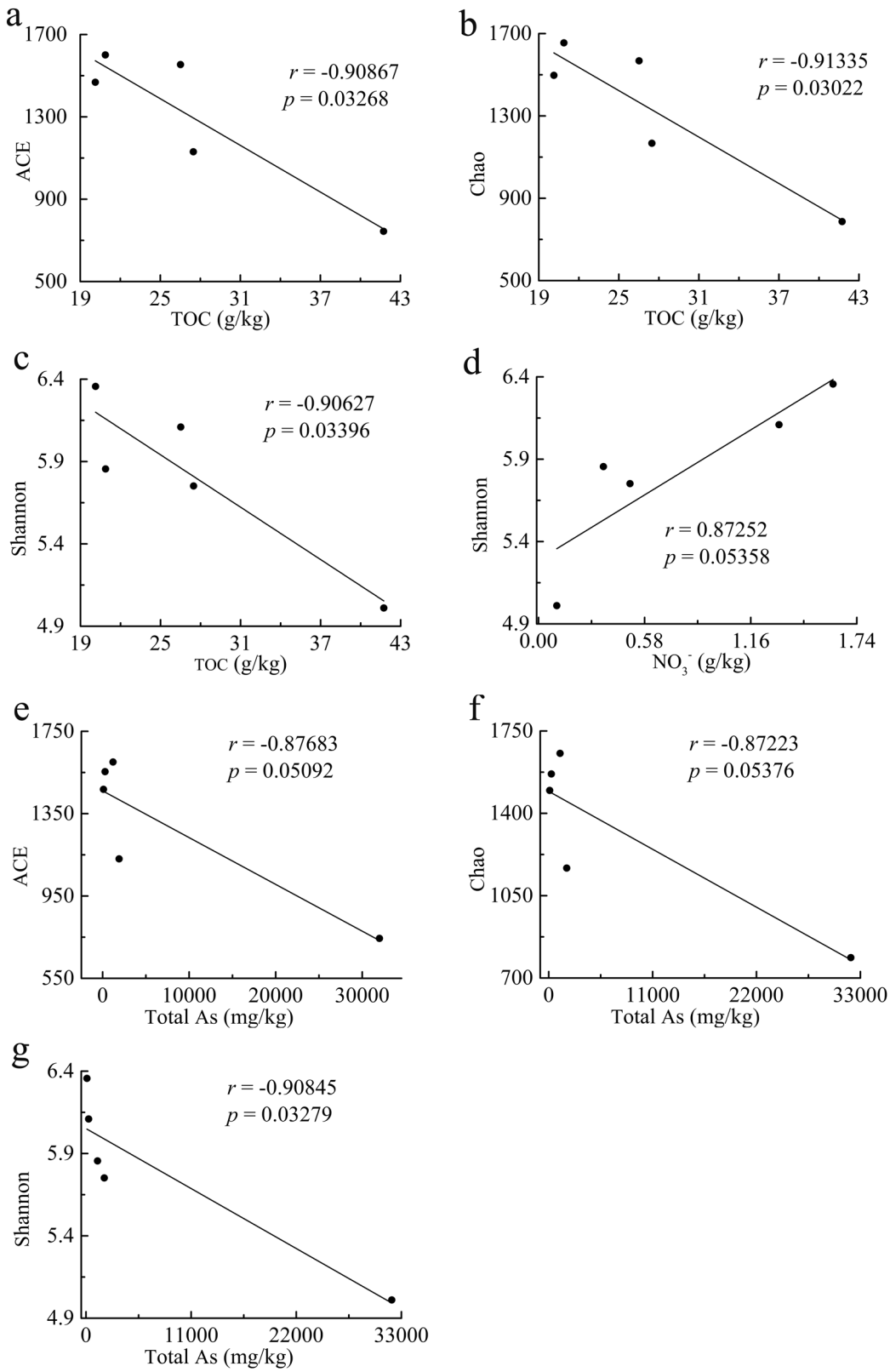

Fig. 6 Correlations between the microbial community diversity and the geochemical parameters

This finding suggests that TOC and total As may also affect the As-methylating activities of the tailing samples.

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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 human participants or animals performed by any of the author.

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