Metagenomic analysis revealed highly diverse microbial arsenic metabolism genes in paddy soils with low-arsenic contents

Ke-Qing Xiao ^{a, b, 1, 2}, Li-Guan Li ^{c, 1}, Li-Ping Ma ^c, Si-Yu Zhang ^{a, b}, Peng Bao ^a, Tong Zhang ^{c, **}, Yong-Guan Zhu ^{a, d, *}

^a State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, 18 Shuangqing Road, Beijing, 100085, China

^b University of Chinese Academy of Sciences, 19A Yuquan Road, Beijing, 100049, China

^c Environmental Biotechnology Laboratory, Department of Civil Engineering, The University of Hong Kong, Hong Kong, China

^d Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, 1799 Jimei Road, Xiamen, 361021, China

ARTICLE INFO

Article history:

Received 15 September 2015

Received in revised form

12 December 2015

Accepted 14 December 2015

Available online 29 December 2015

Handling Editor: Jay Gan.

Keywords:

Metagenomic analysis

Paddy soil

Arsenic metabolism genes

Diversity

ABSTRACT

Microbe-mediated arsenic (As) metabolism plays a critical role in global As cycle, and As metabolism involves different types of genes encoding proteins facilitating its biotransformation and transportation processes. Here, we used metagenomic analysis based on high-throughput sequencing and constructed As metabolism protein databases to analyze As metabolism genes in five paddy soils with low-As contents. The results showed that highly diverse As metabolism genes were present in these paddy soils, with varied abundances and distribution for different types and subtypes of these genes. Arsenate reduction genes (*ars*) dominated in all soil samples, and significant correlation existed between the abundance of *arr* (arsenate respiration), *aio* (arsenite oxidation), and *arsM* (arsenite methylation) genes, indicating the co-existence and close-relation of different As resistance systems of microbes in wetland environments similar to these paddy soils after long-term evolution. Among all soil parameters, pH was an important factor controlling the distribution of As metabolism gene in five paddy soils ($p = 0.018$). To the best of our knowledge, this is the first study using high-throughput sequencing and metagenomics approach in characterizing As metabolism genes in the five paddy soil, showing their great potential in As biotransformation, and therefore in mitigating arsenic risk to humans.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Arsenic (As) is a notoriously poisonous trace element which is ubiquitous in natural environment and can enter into the food chain through various pathways (Cai et al., 2013; Xiao et al., 2012; Zhang et al., 2015; Zhu et al., 2014). Typically, rice can accumulate As from both polluted and unpolluted paddy soils, posing a potential health risk to populations with high rice consumption in China, Southern Asian and certain Latin American areas (Li et al.,

2011; Zhu et al., 2014). Microbial mediated As metabolic processes play a major role in As cycling in the plant-soil-microbe system, including arsenite (As(III)) oxidation, arsenate (As(V)) respiration, As(V) reduction, and As(III) methylation (Lett et al., 2012). Recently, anaerobic As(III) oxidation was found in the haloalkaliphilic bacterium *Alkalilimnicola ehrlichii* strain MLHE-1 and the photosynthetic purple sulfur bacterium *Ectothiorhodospira* sp. strain PHS-1 (Kulp et al., 2008; Zargar et al., 2010), which was suggested to be conducted by a new arsenite oxidase clade, ArxA (Zargar et al., 2012), though no information is presently known on the this enzyme's functioning (van Lis et al., 2013). Through these processes, As speciation and bioavailability are altered, affecting its toxicity, environmental behavior and fate in the environment, and also its accumulation into food crops (such as rice) (Jia et al., 2014, 2013; Stolz and Oremland, 1999).

Arsenic metabolism genes mainly consist of four types: *aio* genes (As(III) oxidation, previously named as *aox/aso/aro*) (Lett et al., 2012), *arr* genes (As(V) respiration) (Malasarn et al., 2004),

* Corresponding author. State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, 18 Shuangqing Road, Beijing, 100085, China.

** Corresponding author.

E-mail addresses: zhangt@hku.hk (T. Zhang), ygzhu@rcees.ac.cn (Y.-G. Zhu).

¹ These authors contributed equally to this work.

² Present address: Center for Geomicrobiology, Department of Bioscience, Aarhus University, Ny Munkegade 114, 8000 Aarhus C, Denmark.

ars genes (As(V) reduction related) (Oremland and Stolz, 2005), and *arsM* genes (As(III) methylation) (Mestrot et al., 2011; Qin et al., 2006). These genes, and their corresponding proteins or their subunits (subtypes) are frequently used as molecular markers for microbial ecology studies (Lever, 2013; Malasarn et al., 2004; Silver and Phung, 2005). Traditionally, degenerate primer sets were designed and used for many PCR-based methods to investigate the distribution and diversity of one or several subtypes of As metabolism genes (Jia et al., 2014, 2013; Schuchmann and Muller, 2014; Silver and Phung, 2005). Jia et al. (2013) for the first time designed specific primers and successfully amplified prokaryotic *arsM* gene in 14 tested soils with wide range of As concentrations, and found that microbes containing *arsM* genes were phylogenetically diverse and widespread. Moreover, their results based on *aioA*, *arsC* and *arrA* highlighted that As uptake by rice is influenced by microbe-mediated As redox processes, which are influenced by root oxygen release and organic matter application (Jia et al., 2014). These techniques are classical but limited due to the bias of the primers and potential nonspecific amplification as well as a lack of available primers sometimes. With the development of high-throughput sequencing techniques and dramatic drop of sequencing prices, diversity analysis based on metagenome becomes feasible, which can target all As metabolism genes and does not rely on the specificity and coverage of the primers used (Cai et al., 2013).

Most As-related studies targeted high-As contaminated environments, while less attention has been paid on natural environments with low-As levels. This study aimed to investigate the distribution, diversity, and abundance of As metabolism genes in typical paddy soils with low-As contents from Southern China. The findings of this study will provide a novel insight into and a better understanding of microbial mediated As metabolism in low-As soil habitats.

2. Material and methods

2.1. Soil sampling and arsenic analysis

Paddy soil samples were collected from five sites: Leizhou in Guangdong Province (LZ), Jiaxing in Zhejiang Province (JX), Yingtan in Jiangxi Province (YT), Gushi of Taoyuan in Hunan Province (TY-G), and Baodongyu of Taoyuan in Hunan Province (TY-B). Soils from the surface (0–20 cm) were taken and divided for DNA extraction and physiochemical analysis. These soils were distinct from each other in soil type, parent material and some other physiochemical properties, as reported previously (Xiao et al., 2014a, 2014b). Total As contents in the soil were detected using method described previously (Sun et al., 2008; Williams et al., 2007; Zhu et al., 2014). Briefly, after air-dried and sieved (mesh size = 150 μ m), soil samples and standards with known As concentrations (1.5–2.5 g) were digested with aqua regia (prepared before use), 60% perchloric acid and 1 M hydrogen chloride successively. Arsenic in the residue was dissolved and diluted by adding Milli-Q water, and measured using ICP-MS (7500a, Agilent Technologies), and the recovery efficiency was $88.87 \pm 0.01\%$.

2.2. DNA extraction and sequencing

DNA was extracted from the soil samples in duplicate using the MoBio PowerSoil kit (MOBIO) according to the manufacturer's protocol. DNA yields of the 10 samples were between 1.0 and 2.5 μ g, as quantified using the Quant-iT Picogreen dsDNA HS assay kit (Invitrogen). Sequencing was performed using Illumina Hiseq 2000 (Illumina) at Majorbio, Inc., Shanghai, China. Raw reads (101 bp in length) were trimmed to remove low quality reads that contained ambiguous nucleotides or had a quality value lower than 20 (Chen

et al., 2013). In total, 750,385,006 clean reads were generated with an average of 75,038,500 reads per sample. Data is available at the NCBI Short Read Archive under accession number SRP039858.

2.3. Bioinformatics analysis

The local BLASTX programs were employed to align trimmed clean reads of each data set against an As metabolism protein database constructed previously (Cai et al., 2013). This database included a total of 17 subdatabases, corresponding to 17 subtypes of As metabolism genes, *aio* genes (*aioX*, *aioS*, *aioR*, *aioB*, *aioA*, *aioC*, and *aioD*), *arr* genes (*arrA* and *arrB*), *ars* genes (*arsR*, *arsD*, *arsA*, *arsB*, *arsC*, *arsH* and *ACR3*) and *arsM*. We used the m 8 output format and e-value cutoffs of $1e^{-5}$ to do the best BLAST search (Mackelprang et al., 2011). The BLASTX outputs against As metabolism protein database were screened by a self-written script to extract alignments of aligned length ≥ 25 aa and identity $\geq 90\%$ (Cai et al., 2009).

Fig. 1. Total arsenic content (a), composition and abundance of arsenic (As) metabolism genes in these five paddy soils, (b) based on abundances (ppm, one read in one million reads), (c) based on percentages (%). Abbreviation: LZ, Leizhou in Guangdong Province; JX, Jiaxing in Zhejiang Province; YT, Yingtan in Jiangxi Province; TY-G, Gushi of Taoyuan in Hunan Province; TY-B, Baodongyu of Taoyuan in Hunan Province, same for the followings.

Fig. 2. Redundancy analysis of soil parameters and 17 subtypes of As metabolism genes.

Based on data from BLAST research and script-screening, the proportions of different types of As metabolism genes were defined as “percentage” (% in “total As metabolism genes sequences”) and “abundance” (ppm, one read in one million reads, in “total meta-genome sequences”), respectively (Yang et al., 2013). The MEGA 5 (Tamura et al., 2011) and PAST (Hammer et al., 2001) local tools were used to construct a phylogenetic tree and perform cluster analysis. BAND program (Heinrich-Salmeron et al., 2011) and MATLAB software (<http://www.mathworks.com/>) were applied to visualize the As metabolism genes abundance profile.

2.4. Statistical analysis

Correlation analyses were carried out using the Pearson correlation method with significance defined at the 0.05 level (SPSS 13.0). Principal component analysis (PCA) was performed based on the abundances of the As metabolism genes subtypes. Additionally, redundancy analysis (RDA) combined with Monte Carlo permutation was conducted to find the relationship between the soil parameters (cf. Table 1 in Xiao et al., 2014a) and the abundance of As metabolism genes using CANOCO 4.5 for Windows (Microcomputer Power) (Xiao et al., 2014a).

3. Results and discussion

3.1. Total arsenic As content in these five soil samples

Total As concentration in all samples except Yingtan (YT) were below the first class standard of environmental quality standards for soils of China (15 mg kg^{-1} , GB 15168-1995), and As concentration in YT was far below the secondary class standard of Environmental Quality Standards for Soils of China (30 mg kg^{-1} , GB 15168-1995), as shown in Fig. 1a. According to the GB 15168-1995, the first class standard (15 mg kg^{-1}) applies to environments like nature reserve with background As concentration, the second class standard (30 mg kg^{-1}) apply to environments like uncontaminated agricultural lands, indicating that our paddy soil samples can be

Fig. 3. Correlation analysis between different types of As metabolism genes.

considered as having low-arsenic level. It was reasonable since all of these samples were taken from rural areas of Southern China, which were generally less exposed to sources like industrial waste or mining drainage (Zhao et al., 2010; Zhu et al., 2014).

3.2. Abundance of As metabolism genes and statistical analysis

The abundance and percentage of As metabolism genes assigned into four types (different metabolism pathways) are shown in Fig. 1b and c. The overall abundance range of As metabolism genes in paddy soil was 80–120 ppm (one read in one million reads), and over 85% of them were As(V) reduction related genes (*ars*) in all soil samples, which are involved in As detoxification processes, such as cytoplasmic As(V) reduction and As(III) efflux (Silver and Phung, 2005). These genes are also prevalent among sediment and activated sludge (Cai et al., 2013), which are generally characterized by low redox potential. As(III) methylation genes (*arsM*) constituted secondary important part of total As metabolism genes (6–9%) among all five paddy soils, and higher than *aio* and *arr* in our samples, implying a strong potential to produce methylated As species, which could be uptake by rice roots and explain why most rice contains unusually high concentrations

Fig. 4. Relative abundance (%) of *aioA* and *arrA* genes in these five paddy soils (heatmap in middle) and phylogenetic analysis using full-length protein sequences derived from AioA and ArrA sub-database (phylogenetic tree on the left and strain source with GenBank ID on the right).

of methylated As species compared with other cereals (Jia et al., 2013). The low abundance of *arr* genes compared with the others detected in the five paddy soils, which has also been confirmed either in paddy soils or As and antimony (Sb) contaminated mine field (Jia et al., 2014, 2013; Luo et al., 2014; Zhang et al., 2015), could be partly ascribed to their specific presence in anaerobic As(V) respiring bacteria (Malasarn et al., 2004; Silver and Phung, 2005). Previous studies showed that total As concentrations in paddy soils correlated positively with the abundance of genes involved in As biotransformation processes (Zhang et al., 2015), and concentrations of different As species in soil solutions have also been revealed good correlations with the abundance of genes responsible for different As biotransformation processes in microcosm experiments (Jia et al., 2014, 2013). Our results showed no significant correlation between total As content and abundance of different types of As metabolism genes (Table S1), which were possibly due to the low As level in these soil samples.

Redundancy analyses (RDA) of 17 As metabolism genes (Fig. S1) and soil parameters (Xiao et al., 2014a) showed that pH was significantly correlated with gene abundance variation among different paddy soil ($p = 0.018$), contributing 52% to the variance (Fig. 2), suggesting that microbially-mediated As metabolic processes in paddy soils were sensitive to pH variation, as suggested previously (Stolz and Oremland, 1999). Actually, the pH value is known to be an important factor in many microbial processes, such as acetogenesis, ammonia oxidation, CO₂ fixation, and methanogenesis, and worked mainly by influencing nutrients or substrates availability and microbial cell physiology (Schuchmann and Muller,

2014; Taconi et al., 2008; Xiao et al., 2014a; Zhang et al., 2012). Significant correlation existed between the abundance of *ars*, *aio*, and *arsM* genes, but not *arr* genes (Fig. 3). Zhang et al. (2015) also revealed the strong correlation of *arsC* and *arsM* gene copies based on quantitative PCR, and suggested that similar compositions of microbial communities are involved in As(V) detoxification reduction and As(III) methylation in paddy soil. Considering that *ars*, *aio*, and *arsM* genes are separately involved in cytoplasmic As(V) reduction, As(III) oxidation, and As(III) methylation processes, the significant correlation between the abundances of *ars*, *aio*, and *arsM* gene indicated the co-existence and close-relation of these different As resistance systems in microbes in paddy soil (Jia et al., 2014, 2013; Zhang et al., 2015; Zhu et al., 2014).

3.3. Distribution, diversity, and abundance of *aioA* and *arrA* genes

To investigate the potential As redox in the environment, *aioA* has been proposed as a valuable functional marker gene responsible for As(III) oxidation and *arrA* as a reliable marker gene for As(V) respiration reduction (Malasarn et al., 2004; Quémeuneur et al., 2008; Silver and Phung, 2005). AioA and ArrA proteins are both molybdenum-containing enzymes in the dimethyl sulfoxide (DMSO) reductase family (Oremland and Stolz, 2005; Silver and Phung, 2005), sharing similar amino acid length and structural characteristic while carrying out completely distinct functions. The diversity, distribution and abundance of both genes were further mined here. As shown in Fig. 4, they were divided into two clades clearly, displaying remarkable heterogeneous abundance profile

Fig. 5. Relative abundance (%) of *arsC* genes in these five paddy soils (heatmap in middle) and phylogenetic analysis of top 40 *arsC* sequences using full-length protein sequences derived from ArsC sub-database (phylogenetic tree on the left and strain source with GenBank ID on the right).

among the 5 samples, as a few of the *aioA* and *arrA* genes distributed in more than three environments (e.g. *Ralstonia* sp. 22 (ACX69823)) while some specifically existed in only one environment (e.g. *Thiomonas intermedia* K12 (YP_001098817)). Researchers have discovered general consistence between *aioA* and 16S rRNA gene phylogeny in particular field, as has been underlined for other functional marker genes (Inskeep et al., 2007; Jackson and Dugas, 2003; Klein et al., 2001; Wagner et al., 1998). The most abundant *aioA* sequence in all five studied samples was derived from *Nitrobacter hamburgensis* X14 (YP_571843). Among the 26 detected *aioA* sequences, the majority were from *Burkholderiales* and *Rhizobiales* at order level, which were also the two main microbial communities in rhizospheric bacteria and contributed significantly to the oxidation of As(III) in soils under both aerobic and anaerobic conditions (Jia et al., 2014; Rhine et al., 2005). In paddy TY-B, *arrA* genes were detected with the highest diversity but with the least abundance, and the lowest diversity was found in paddies LZ and YT. In addition, the shared dominant *arrA* gene sequences in all 5 samples were derived from *Opitutus terraet* PB90-1 (YP_001818350). Contrary to *aioA* genes, high diversity (including *Firmicutes*, *Chlamydiae*, *Proteobacteria*, *Deferribacteres* and *Chrysiogenetes*) of As(V)-respiring systems in paddy soil appeared in phylogenetic analysis of the *arrA* gene sequences. From an evolutionary perspective, it is intriguing that As(V)-respiring bacteria are phylogenetically diverse while *arrA* gene sequences are well-reserved at the same time (Malasarn et al., 2004), one speculation could be the deceased

evolution since the Great Oxygenation Event of Earth, which resulted in increased oxygen concentration in the atmosphere and evolution of aerobic respiration (Schirmer et al., 2013).

3.4. Distribution, diversity, and abundance of *arsC* genes

The well-studied As(V) reducing pathways typically include detoxification reduction (ArsC) and dissimilatory respiration (ArrA/B). ArrA/B is either membrane-bound or free in periplasm, while ArsC located in cytoplasm could reduce As(V) within cytoplasmic membrane and subsequently excrete As(III) by ArsAB efflux pump (Oremland and Stolz, 2005). Given the habitat type as paddy soil here, As(V) detoxification functioning under both aerobic and anaerobic conditions (Bhattacharjee and Rosen, 2007; Silver and Phung, 2005) appears to be more widespread compared with As(V) respiration which is limited by anaerobic conditions. As shown in Fig. 1, the abundance of *ars* genes ranged from 81.11 to 103.82 ppm (one read in one million reads), far more than *arr* genes ranging from 0.64 to 1.30 ppm (one read in one million reads), which was similar to Cai et al. (2013), and could be ascribed to the versatile lifestyle of As(V) reducing bacteria (Silver and Phung, 2005). To gain a deep perspective of As(V) reduction, the critical gene *arsC* in As(V) reduction was explored. Clustering based upon abundance of *arsC* indicated the closest proximity between paddies JX and YT, which was grouped with paddy LZ and further clustered with paddy TY-G., and finally with paddy TY-B (Fig. S2). Whole

Fig. 6. Relative abundance (%) of *arsM* genes from five paddy soil samples (heatmap in middle) and phylogenetic analysis using full-length protein sequences derived from ArsM sub-database (phylogenetic tree on the left and strain source with GenBank ID on the right).

abundance profile in Figure S2 revealed obvious inhomogeneous distribution among different gene sequences, with ~80% of total *arsC* gene abundance in the five samples was attributed to top 40 abundant sequences. Therefore, top 40 *arsC* sequences were chosen for following phylogenetic analysis, showing a broad phylogenetic distribution of *arsC* genes (Fig. 5), while 45% and 8% of the top 40 *arsC* genes presented were from *Rhizobiales* and *Burkholderiales*—also two major groups in *aioA* phylogeny (Fig. 4), and dominating in rice root rhizospheric bacteria (Jia et al., 2014; Rhine et al., 2005). Moreover, the most abundant *arsC* gene was from *Afpia* sp. 1NLS2 (EF150814), accounting for 27.11% of total *arsC* gene sequences detected. While the *aioA* tree generally reflect the true 16S rDNA phylogeny, the inconsistency (Fig. 5) between the overall phylogeny of 16S rDNA and *arsC* seemed common (Jackson and Dugas, 2003; Zhang et al., 2015), which might be attributed to the role of multiple-cases of chromosomal-plasmid exchange and following horizontal gene transfer happening simultaneously in microbial evolution (Jackson and Dugas, 2003).

3.5. Distribution, diversity, and abundance of *arsM* genes

The tree in Fig. 6 showed that the *arsM* sequences hosted by indigenous microbial community were branching into diverse phyla, mainly composed of *Proteobacteria*, *Firmicutes*, and *Bacteroidetes*, suggesting the genes encoding ArsM were widespread in paddy soil (Jia et al., 2013). The highest genotype diversity was observed in paddy LZ, while the lowest was in paddies YT and TY-G. The sequence contributing most to abundance in paddies JX, YT, TY-

G and TY-B was from *Candidatus Solibacter usitatus* Ellin6076 (YP_825656), with the exception of paddy LZ from *Rhodopseudomonas palustris*, which was confirmed to produce volatile trimethylarsine (TMAs) as the final product of As(III) methylation reaction (Mestrot et al., 2011). Compared with other investigated environmental sites of similar As background level (mean 3.65%) (Cai et al., 2013), relative abundance of *arsM* gene (mean 8.07%) was higher here, which could be mainly attributed to the unique biogeochemical conditions in paddy soil, such as oxic/anoxic alternate due to root oxygen release, organic matter input from inside (root) and outside (straw), and those abundant autotrophic sulfate-reducing bacteria as well as methanoarchaea, favoring As mobilization and synergy between different microbial groups (Jia et al., 2013; Zhang et al., 2015). The conversion between the more toxic As(III) and the less toxic As(V) cannot remove As from soils, while As(III) methylation mediated by *arsM* and coding protein and subsequent volatilization is an important pathway for As removal from soils and sediments, indicating a high potential for paddy soil to remove As, which has been verified by both lab and filed experiments (Huang et al., 2012; Mestrot et al., 2011).

3.6. Possible evolutionary history affecting the abundance and diversity of As metabolism genes

In all five paddy soils with low-As content, most of the detected genes involved in As metabolic processes were responsible for As detoxification, such as *aio*, *ars*, *arsM* (Bhattacharjee and Rosen, 2007; Silver and Phung, 2005; Zhu et al., 2014), indicating the

prevalence of As resistance in microbes. Since As(III) was the dominant As species due to the reducing conditions in primordial atmosphere, early organisms would firstly evolve detoxification mechanisms to cope with As(III) (Rosen, 2002), which might contribute to the most abundant genes involved in As(III) efflux (*arsB* and *ACR3*) detected in the five paddy soils even with low-As level (Fig. S1). The mechanisms to cope with As(V) might have evolved to use existing As(III) extrusion systems later when the atmosphere became oxidized (Rosen, 2002), for example, the cytoplasmic reduction of As(V) to As(III) mediated by *arsC* detected in the soils (Fig. 1) followed by As(III) efflux as the detoxification process. For anaerobic As(V) respiration process mediated by *arr* genes, in which As(V) is used as the anaerobic terminal electron acceptors, though it is still being debated whether it would occur preceding aerobic oxygen-utilizing respiratory electron transport chains. However, the anaerobic bacteria should arise from prebiotic forms hundreds of millions of years before the oxygen respiration first occurs (Silver and Phung, 2005).

4. Conclusion

Our study provided an overall picture of genes related to As metabolic processes in paddy soil by high-throughput sequencing and metagenomics approach. Arsenic metabolism genes were ubiquitous, abundant, and associated with diverse microbes even in low-As environments, and statistical analysis showed pH as an important factor controlling their distribution in paddy soil. *ars* dominated in all paddy soil samples, and was well correlated with *aio* and *arsM*. These results indicate a combinational effect of evolution and selection on As metabolism in microbes under different environmental conditions.

Acknowledgments

This work was supported by Natural Science Foundation of China Grant 41090282 and Key Projects of Natural Science Foundation of China Grant 41990280.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.envpol.2015.12.023>.

References

- Bhattacharjee, H., Rosen, B.P., 2007. *Molecular Microbiology of Heavy Metals*. Springer-Verlag Press, Berlin, Germany.
- Cai, L., Liu, G., Rensing, C., Wang, G., 2009. Genes involved in arsenic transformation and resistance associated with different levels of arsenic-contaminated soils. *BMC Microbiol.* 9, 4.
- Cai, L., Yu, K., Yang, Y., Chen, B.-w., Li, X.-d., Zhang, T., 2013. Metagenomic exploration reveals high levels of microbial arsenic metabolism genes in activated sludge and coastal sediments. *Appl. Microbiol. Biotechnol.* 97, 9579–9588.
- Chen, B., Yang, Y., Liang, X., Yu, K., Zhang, T., Li, X., 2013. Metagenomic profiles of antibiotic resistance genes (ARGs) between human impacted estuary and deep ocean sediments. *Environ. Sci. Technol.* 47, 12753–12760.
- Hammer, Ø., Harper, D., Ryan, P., 2001. Past: paleontological statistics software package for education and data analysis. *Paleontol. Electrónica* 4, 1–9. http://paleo-electronica.org/2001_1/past/issue1_01.html.
- Heinrich-Salmeron, A., Cordi, A., Brochier-Armanet, C., Halter, D., Pagnout, C., Abbaszadeh-fard, E., Montaut, D., Seby, F., Bertin, P.N., Bauda, P., Arsène-Ploetze, F., 2011. Unsuspected diversity of arsenite-oxidizing bacteria as revealed by widespread distribution of the *aoxB* gene in prokaryotes. *Appl. Environ. Microbiol.* 77, 4685–4692.
- Huang, H., Jia, Y., Sun, G.-X., Zhu, Y.-G., 2012. Arsenic speciation and volatilization from flooded paddy soils amended with different organic matters. *Environ. Sci. Technol.* 46, 2163–2168.
- Inskeep, W.P., Macur, R.E., Hamamura, N., Warelou, T.P., Ward, S.A., Santini, J.M., 2007. Detection, diversity and expression of aerobic bacterial arsenite oxidase genes. *Environ. Microbiol.* 9, 934–943.
- Jackson, C.R., Dugas, S.L., 2003. Phylogenetic analysis of bacterial and archaeal *arsC* gene sequences suggests an ancient, common origin for arsenate reductase. *BMC Evol. Biol.* 3, 18–18.
- Jia, Y., Huang, H., Chen, Z., Zhu, Y.-G., 2014. Arsenic uptake by rice is influenced by microbe-mediated arsenic redox changes in the rhizosphere. *Environ. Sci. Technol.* 48, 1001–1007.
- Jia, Y., Huang, H., Zhong, M., Wang, F.-H., Zhang, L.-M., Zhu, Y.-G., 2013. Microbial arsenic methylation in soil and rice rhizosphere. *Environ. Sci. Technol.* 47, 3141–3148.
- Klein, M., Friedrich, M., Roger, A.J., Hugenholtz, P., Fishbain, S., Abicht, H., Blackall, L.L., Stahl, D.A., Wagner, M., 2001. Multiple lateral transfers of dissimilatory sulfite reductase genes between major lineages of sulfate-reducing prokaryotes. *J. Bacteriol.* 183, 6028–6035.
- Kulp, T.R., Hoeft, S.E., Asao, M., Madigan, M.T., Hollibaugh, J.T., Fisher, J.C., Stolz, J.F., Culbertson, C.W., Miller, L.G., Oremland, R.S., 2008. Arsenic(III) fuels anoxygenic photosynthesis in hot spring biofilms from Mono Lake, California. *Science* 321, 967–970.
- Lett, M.-C., Muller, D., Lièvremon, D., Silver, S., Santini, J., 2012. Unified nomenclature for genes involved in prokaryotic aerobic arsenite oxidation. *J. Bacteriol.* 194, 207–208.
- Lever, M.A., 2013. Functional gene surveys from ocean drilling expeditions – a review and perspective. *FEMS Microbiol. Ecol.* 84, 1–23.
- Li, G., Sun, G.-X., Williams, P.N., Nunes, L., Zhu, Y.-G., 2011. Inorganic arsenic in Chinese food and its cancer risk. *Environ. Int.* 37, 1219–1225.
- Luo, J., Bai, Y., Liang, J., Qu, J., 2014. Metagenomic approach reveals variation of microbes with arsenic and antimony metabolism genes from highly contaminated soil. *PLoS One* 9, e108185.
- Mackelprang, R., Waldrop, M.P., DeAngelis, K.M., David, M.M., Chavarria, K.L., Blazewicz, S.J., Rubin, E.M., Jansson, J.K., 2011. Metagenomic analysis of a permafrost microbial community reveals a rapid response to thaw. *Nature* 480, 368–371.
- Malasarn, D., Saltikov, C.W., Campbell, K.M., Santini, J.M., Hering, J.G., Newman, D.K., 2004. *arrA* is a reliable marker for As(V) respiration. *Science* 306, 455.
- Mestrot, A., Feldmann, J., Krupp, E.M., Hossain, M.S., Roman-Ross, G., Meharg, A.A., 2011. Field fluxes and speciation of arsenites emanating from soils. *Environ. Sci. Technol.* 45, 1798–1804.
- Oremland, R.S., Stolz, J.F., 2005. Arsenic, microbes and contaminated aquifers. *Trends Microbiol.* 13, 45–49.
- Qin, J., Rosen, B.P., Zhang, Y., Wang, G., Franke, S., Rensing, C., 2006. Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite S-adenosylmethionine methyltransferase. *Proc. Natl. Acad. Sci. U. S. A.* 103, 2075–2080.
- Quéméneur, M., Heinrich-Salmeron, A., Muller, D., Lièvremon, D., Jauzein, M., Bertin, P.N., Garrido, F., Joulain, C., 2008. Diversity surveys and evolutionary relationships of *aoxB* genes in aerobic arsenite-oxidizing bacteria. *Appl. Environ. Microbiol.* 74, 4567–4573.
- Rhine, E.D., Garcia-Dominguez, E., Phelps, C.D., Young, L.Y., 2005. Environmental microbes can speciate and cycle arsenic. *Environ. Sci. Technol.* 39, 9569–9573.
- Rosen, B.P., 2002. Biochemistry of arsenic detoxification. *FEBS Lett.* 529, 86–92.
- Schirmer, B.E., de Vos, J.M., Antonelli, A., Bagheri, H.C., 2013. Evolution of multicellularity coincided with increased diversification of cyanobacteria and the great oxidation event. *Proc. Natl. Acad. Sci.* 110, 1791–1796.
- Schuchmann, K., Muller, V., 2014. Autotrophy at the thermodynamic limit of life: a model for energy conservation in acetogenic bacteria. *Nat. Rev. Microbiol.* 12, 809–821.
- Silver, S., Phung, L.T., 2005. Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. *Appl. Environ. Microbiol.* 71, 599–608.
- Stolz, J.F., Oremland, R.S., 1999. Bacterial respiration of arsenic and selenium. *FEMS Microbiol. Rev.* 23, 615–627.
- Sun, G.-X., Williams, P.N., Carey, A.-M., Zhu, Y.-G., Deacon, C., Raab, A., Feldmann, J., Islam, R.M., Meharg, A.A., 2008. Inorganic arsenic in rice bran and its products are an order of magnitude higher than in bulk grain. *Environ. Sci. Technol.* 42, 7542–7546.
- Taconi, K.A., Zappi, M.E., Todd French, W., Brown, L.R., 2008. Methanogenesis under acidic pH conditions in a semi-continuous reactor system. *Bioresour. Technol.* 99, 8075–8081.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28, 2731–2739.
- van Lis, R., Nitschke, W., Duval, S., Schoepp-Cothenet, B., 2013. Arsenics as bio-energetic substrates. *Biochim. Biophys. Acta (BBA) Bioenergetics* 1827, 176–188.
- Wagner, M., Roger, A.J., Flax, J.L., Brusseau, G.A., Stahl, D.A., 1998. Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration. *J. Bacteriol.* 180, 2975–2982.
- Williams, P.N., Villada, A., Deacon, C., Raab, A., Figuerola, J., Green, A.J., Feldmann, J., Meharg, A.A., 2007. Greatly enhanced arsenic shoot assimilation in Rice leads to elevated grain levels compared to wheat and barley. *Environ. Sci. Technol.* 41, 6854–6859.
- Xiao, K.-Q., Bao, P., Bao, Q.-L., Jia, Y., Huang, F.-Y., Su, J.-Q., Zhu, Y.-G., 2014a. Quantitative analyses of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) large-subunit genes (*cbhL*) in typical paddy soils. *FEMS Microbiol. Ecol.* 87, 89–101.
- Xiao, K.-Q., Nie, S.-A., Bao, P., Wang, F.-H., Bao, Q.-L., Zhu, Y.-G., 2014b. Rhizosphere effect has no effect on marker genes related to autotrophic CO₂ fixation in paddy soils? *J. Soils Sediments* 14, 1082–1087.

- Xiao, K.-Q., Wang, L., Bai, R., Zhang, S., Yin, X., 2012. Arsenic biotransformation in cyanobacterium *Synechocystis* sp. PCC 6803 under different phosphate regimes. *Fresenius Environ. Bull.* 21, 2551–2557.
- Yang, Y., Li, B., Ju, F., Zhang, T., 2013. Exploring variation of antibiotic resistance genes in activated sludge over a four-year period through a metagenomic approach. *Environ. Sci. Technol.* 47, 10197–10205.
- Zargar, K., Conrad, A., Bernick, D.L., Lowe, T.M., Stolz, V., Hoefft, S., Oremland, R.S., Stolz, J., Saltikov, C.W., 2012. ArxA, a new clade of arsenite oxidase within the DMSO reductase family of molybdenum oxidoreductases. *Environ. Microbiol.* 14, 1635–1645.
- Zargar, K., Hoefft, S., Oremland, R., Saltikov, C.W., 2010. Identification of a novel arsenite oxidase gene, *arxA*, in the haloalkaliphilic, arsenite-oxidizing bacterium *Alkalilimnicola ehrlichii* strain MLHE-1. *J. Bacteriol.* 192, 3755–3762.
- Zhang, L.-M., Hu, H.-W., Shen, J.-P., He, J.-Z., 2012. Ammonia-oxidizing archaea have more important role than ammonia-oxidizing bacteria in ammonia oxidation of strongly acidic soils. *ISME J.* 6, 1032–1045.
- Zhang, S.Y., Zhao, F.J., Sun, G.X., Su, J.Q., Yang, X.R., Li, H., Zhu, Y.G., 2015. Diversity and abundance of arsenic biotransformation genes in paddy soils from southern China. *Environ. Sci. Technol.* 49, 4138–4146.
- Zhao, F.-J., McGrath, S.P., Meharg, A.A., 2010. Arsenic as a food chain contaminant: mechanisms of plant uptake and metabolism and mitigation strategies. *Annu. Rev. Plant Biol.* 61, 535–559.
- Zhu, Y.-G., Yoshinaga, M., Zhao, F.-J., Rosen, B.P., 2014. Earth abides arsenic biotransformations. *Annu. Rev. Earth Planet. Sci.* 42, 443–467.