Microbial transformation of arsenic in Bengal floodplain

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ABSTRACT: Methylation is an important biotransformation process that limits the toxicity of arsenic (As) in soil. Here we investigated how geomorphology and paddy management influences As speciation, and the abundance and diversity of the *arsM* gene responsible for bacterial methylation of arsenic. Soil samples collected from paddy and non-paddy fields of Holocene and Pleistocene regions of Bangladesh were incubated under anaerobic conditions to identify how these treatments affected As speciation in soil solution as well as to investigate the changes in relative *arsM* copy no and diversity in soil. The Holocene soil had higher concentration of soil solution arsenic species (inorganic arsenic, dimethylarsinic acid (DMA), trimethylarsenic oxide (TMAO)), with qPCR showing higher copy numbers of both 16S and *arsM* in Holocene soil compared to Pleistocene soil. Lower soil Eh may explain the higher *arsM* copy number in Holocene soil, with arsenic methylation known to be increased under anaerobic conditions. The higher pH in Holocene soil may also explain the increase in 16S copy number, with bacteria known to be less abundant in acidic soils. Further to that amplicon sequencing showed an increased species richness (chao1) and diversity (Simpson) for both 16S and *arsM* in Holocene compare to Pleistocene soil. PiCrust analysis of the 16S amplicon results showed the presence of arsenic metabolism related genes some of which were increased in Holocene compared to Pleistocene soil. The results showed that presence of As soil chemistry strongly correlates with arsenic transformation and copy number of arsenic metabolizing genes and bacterial as well as *arsM* gene diversity.

1 INTRODUCTION

Paddy soils in the Holocene regions of Bangladesh is elevated in inorganic arsenic (iAs), while Pleistocene soils are lower in iAs, most likely due to its and paddy management practices, such as flooding of irrigation of Holocene soils with As contaminated waters (Lu, *et al.*, 2009). Several studies found that, Holocene floodplain contained higher total As compared to Pleistocene terrace soil (Chowdhury *et al.*, 2017; Lu, *et al.*, 2009; William *et al.*, 2011). This is because, Holocene soils are less weathered and the parent material are still undergoing subsequent weathering process while the Pleistocene soils are highly weathered (William *et al.*, 2011). As methylation is an important biotransformation process that catalyzes toxic iAs to less toxic methylated As species such as MMA (monomethylarsonic acid), DMA and TMAO. The rate of methylation depends on As species and the abundance and diversity of *arsM* gene in soil (Jia *et al.*, 2014; Zhao *et al.*, 2013). In this study, we investigated the effect of geomorphology and paddy management system on As speciation and diversity of *arsM* gene in Bangladeshi soil.

2 MATERIAL AND METHODS

2.1 Soil sample collection incubation

A total of 20 soil samples were collected from paddy and adjacent non-paddy fields across Holocene

(Araihazar Upazila) and Pleistocene regions (Madhupur Upazila) of Bangladesh. Field moist soils were incubated for 2 weeks under anaerobic condition to stimulate the activity of microbial population in soil. After two weeks, soil pore water and soils were collected and both soil and pore water immediately frozen and stored at -20° until subsequent analysis. pH and Eh was recorded in replicated microcosms by inserting an Eh/pH meter into the soil at harvest. Arsenic species in 0.4-micron Millipore membrane filtered soil pore waters (iAs, DMA and TMAO) were determined using a Dionex IC chromatographic system interfaced with ICP-MS (ICS-5000 DC, Thermo Scientific).

2.2 DNA extraction, qPCR and Amplicon sequencing

Soil DNA was extracted using the Powerlyzer[®] Powersoil[®] DNA isolation kit (MOBIO Laboratories, Inc.) following the manufacturer's instructions. Soil DNA was amplified for bacterial 16S and *arsM* gene using specific primer pairs 16S_1369F/16S_1492R (Jia *et al.*, 2014) and *arsM*F1/*arsM*R2 (Jia *et al.*, 2013), respectively and relative copy no was measured by qPCR on the Eppendorf mastercycler (Realplex⁴, Hamburg, Germany) using SYBR green (PrimerDesign, USA). Following purification and quantification soil DNA samples were sent to Centre for Genomic Research institute, University of Liverpool for amplicon sequencing of the 16S rDNA and *arsM* gene on the Illumina Miseq using 250 paired end sequencing.

Figure 1. Linear Relationships for bacterial 16S (a) and arsM (b) gene copy no. versus soil As, soil solution As species, pH and Eh. The line on each graph is the regression line for this parameter. “hn” represents Holocene non-paddy “hp” represents Holocene paddy, “pn” Pleistocene non-paddy, “pp” Pleistocene paddy.

Sequences were analyzed using QIIME version 1.8 (Caporaso *et al.*, 2010) followed by statistical analysis in R.

2.3 Characterization of soils

The soil samples were oven dried (70°C for 48 h) to constant weight and analyzed for t-As using ICP-MS.

2.4 Statistical analysis

Statistical analysis was performed in Minitab version 16 (Minitab, PA, USA) and Graphpad prism7.

Figure 2. Alpha diversity (Richness and Simpson evenness) in bacterial 16S and arsM gene. Significant differences ($p < 0.05$) was observed for geomorphology (Holocene/Pleistocene).

3 RESULTS AND DISCUSSION

3.1 Soil solution As species, pH, Eh and qPCR

The concentration of soil As, soil solution As species and relative copy no of bacterial 16S and arsM gene were higher in Holocene soils compared to Pleistocene soils. The linear relationship between bacterial 16S and arsM gene copy no with chemical parameters (soil As, soil solution As species, pH and Eh) is shown in Figure 1.

The strong linear relationships of 16S and arsM gene copy with soil pH and Eh suggests that, these are the key factors that regulating the DNA copy no and As speciation in soil. High redox potential and acidic pH in Pleistocene soils leads to lower arsM gene copy no in that soils. Because arsM gene is redox sensitive and more active under anaerobic condition (Jia *et al.*, 2013, 2014). In contrast, no significant Paddy management effect was observed in this study. The Pleistocene soils either from paddy and non-paddy soils were less variable in different fields while the Holocene non-paddy soils were more variable.

3.2 Diversity of Bacterial 16S and arsM gene

Alpha diversity measures showed higher species richness (chao1) and overall diversity (Simpson) for both 16S and arsM gene in Holocene compared to Pleistocene soil. While this effect was significant for both 16S and arsM, it was more pronounced in arsM gene (Fig. 2).

4 CONCLUSION

Overall, the concentration of soil As, pore water As species and the abundance of arsM and other As metabolism related genes was shown to vary in two different geomorphic units of Bangladesh, with chemical

and biological parameters correlating. Future investigations will focus on metagenomics analysis to further explore these results.

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