Effect of humic acid on microbial arsenic reduction in anoxic paddy soil

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ABSTRACT: In this study, humic acid (HA) was extracted from peat soil and added to an arsenic-contaminated paddy soil for microcosm experiments to investigate its effect on microbial As(V) reduction in the anoxic paddy soil. The concentration of dissolved As(III) with amendment of HA increased gradually over time and, at the end of the incubation, reached 4.2 mg L^{-1} , which is three times that of the control without amendment. Total RNAs were extracted to profile the potentially active microbial communities and suggested that HA increased the abundance of *Variovorax*, *Azoarcus*, and *Anaeromyxobacter* sp. relative to the control. Transcriptions of the respiratory As(V)-reducing gene *arrA* (10^6 copies mL^{-1}) were significantly higher than those of the cytoplasmic As(V)-reducing gene *arsC* (10^4 copies mL^{-1}). The addition of HA increased the transcripts of *arsC* only during Day 2–6, but increased those of total bacteria and *arrA* during the whole time-course incubation. Our results indicated that the HA extracted from soil enhanced As(V) reduction primarily via up-regulated expression of respiratory As(V)-reducing gene.

1 INTRODUCTION

Arsenic (As) in paddy fields is of great concern due to its high bioavailability to rice and subsequent bioaccumulation in human via dietary intake of rice. Flooding conditions in paddy fields can enhance As(V) reduction, driven by cytoplasmic As(V)-reducing and respiratory As(V)-reducing microorganisms. Humic substances make up the largest single class of dissolved organic matter in the natural environment and can significantly influence microorganism activity and arsenic fate in soils. However, little is known about its effect on microbial As(V) reduction in anoxic paddy soil. In this study, humic acid (HA) was extracted and added to arsenic-contaminated paddy soil to investigate its effect on arsenic transformation, the associated microbial community and transcription levels of As(V)-reducing genes.

2 EXPERIMENTAL METHODS

2.1 Microcosm experiment and Arsenic speciation

The arsenic-contaminated soil was collected from a paddy field in Shantou City (Guangdong Province, PRC). HA was extracted from a peat soil (View Sino International Ltd) using the standard procedure described by International Humic Substances Society (Swift, 1996), and then freeze-dried before use.

Anaerobic microcosm experiments were conducted in serum vials with 70 mL $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ solution (30 mM, pH 7.3), 7 g (wet weight) paddy soil, 1 mL L^{-1} trace element solution, 1 mL L^{-1} vitamin solution with and without 1% HA at 30°C in an anaerobic chamber in the dark. At intervals, dissolved As(III)/As(V) was determined from the supernatant of each culture using atomic fluorescence spectroscopy (SA-20, Jitian

Inc., Beijing, China), and the pellets were used for RNA extraction.

2.2 RNA extraction and microbial community characterization

Total RNA was extracted using a MoBio PowerSoil™ Total RNA Isolation Kit (Mio Bio Laboratories, USA). The RNA was reverse transcribed using a PrimeScript RT reagent kit with gDNA Eraser (Takara, Shiga, Japan). The cDNA samples were amplified with primers specific to V4 region of 16S rRNA gene using primers 515F and 806R for further high-throughput sequencing. Data from 16S rRNA amplicon libraries were processed using the Quantitative Insights Into Microbial Ecology (QIIME 1.8.0) toolkit.

2.3 Transcription and statistical analyses

The cDNA was amplified with primers specific to the *arsC* gene using primers amlt-42-F/amlt-376-R and smrc-42-F/smrc-376R. PCR amplification of the *arrA* gene was achieved with primers *arrA*-CV1F/*arrA*-CV1R. Quantification of transcripts of 16S rRNA, *arrA* and *arsC* genes were performed on an iQ™ 5 Multicolor Real-Time PCR Detection System (Bio-Rad Laboratories, USA) using SYBR Green I detection method. Statistical significance of differences between HA amended and control microcosms was determined via one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests in SPSS 22.0, and with $P < 0.05$ considered to be statistically significant.

3 RESULTS AND DISCUSSION

3.1 Arsenic transformation

At Day 0, concentrations of dissolved As(III) were very low ($<0.001 \text{ mg L}^{-1}$) in both HA-amended and control

microcosms. In the control without amendment, dissolved As(III) began to increase after Day 6 and reached $1.7 \pm 0.1 \text{ mg L}^{-1}$ at the end of the incubation (Day 30). With the HA amendment, the concentrations of dissolved As(III) increased after Day 0 and at the end were $4.2 \pm 0.2 \text{ mg L}^{-1}$, which is three times that of the control. Concentrations of dissolved As(V) showed a similar trend in both HA-amended and control treatments: firstly increasing from $0.2 \pm 0.1 \text{ mg L}^{-1}$ on Day 0 to $0.4 \pm 0.1 \text{ mg L}^{-1}$ on Day 6, and then decreasing over time during the rest of the incubation. These results indicated that As(V) from the solid phase could be released into soil solution and reduced to As(III) in the soil without any amendment, while the addition of HA could facilitate the reduction of As(V) (mainly in solid phase) resulting in the enhancement of As(III) release into the soil solution.

3.2 Composition of active bacterial communities

In both HA-amended and control treatments, *Variovorax* followed by *Azoarcus* were the most abundant species during the incubation. The abundance of *Variovorax* sp. in the control increased from 1–8% during Day 0–2 to 20–22% during the rest of incubation, suggesting that *Variovorax* sp. could be stimulated in anoxic paddy soil without any amendment. In contrast, during the whole incubation time, the abundance of *Variovorax* maintained a high level in the HA-amended microcosm (17–28%). The abundance of *Azoarcus* sp. in the HA-amended treatment was 6–10% during Day 0–2, which was higher than those in the control (0–4%). However, its abundance in both the HA-amended and control treatments decreased in a similar manner as time elapsed during the rest of incubation. Compared with control (<1%), HA had a specific stimulation on the abundance of *Anaeromyxobacter* and its abundance increased gradually over time from $1 \pm 0.03\%$ on Day 0 to $4 \pm 0.1\%$ on Day 30.

V. paradoxus has been reported to be resistant to many metals/metalloids including arsenic (Belimov *et al.*, 2005), and the *ars* operon has been found in the whole-genome of *V. paradoxus* S110 (Han *et al.*, 2011). Some *V. paradoxus* can grow with HA as the sole carbon source (Filip & Bielek, 2002). *Azoarcus* sp. has not shown any ability of As(V) reduction so far, but its genome analysis reveals the existence of genes related to the *ars* operon (Faoro *et al.*, 2017). *Anaeromyxobacter* sp. has been frequently detected in arsenic-contaminated areas and *Anaeromyxobacter* sp. PSR-1 can use As(V) as electron acceptor for respiration (Kudo *et al.*, 2013). Our results suggested that both *Variovorax* and *Azoarcus* sp. were the dominant indigenous microorganisms in the anoxic paddy soil, and HA addition only stimulated the *Variovorax* and *Azoarcus* sp. at the beginning of incubation, but stimulated the *Anaeromyxobacter* sp. at the later stages of incubation.

3.3 Transcription of functional genes

Transcriptions of total bacteria increased rapidly from 0.1×10^8 copies mL^{-1} on Day 0 to 10×10^8 and

5×10^8 copies mL^{-1} on Day 2 in the HA-amended and control treatments, respectively, and then decreased over time to 7×10^8 and 2×10^8 copies mL^{-1} on Day 30. The transcriptions of the *arrA* gene in the control were constant at 0.2×10^6 – 0.7×10^6 copies mL^{-1} during Day 2–30, while those in the HA-amended treatment increased gradually from 0.4×10^6 copies mL^{-1} on Day 2 to 2×10^6 copies mL^{-1} on Day 30. The differences of transcriptions of total bacteria and *arrA* genes between these two treatments were significant ($P < 0.05$). However, transcriptions of *arsC* gene (3.3×10^4 and 4×10^4 copies mL^{-1}) in the HA-amended treatment were significantly higher than those (2×10^4 and 1.5×10^4 copies mL^{-1}) in the control only on Day 2 and Day 6, respectively.

4 CONCLUSIONS

The HA extracted from peat soil can significantly facilitate the reduction of As(V) and release of As(III) into paddy soil solution, probably due to the increase in abundance of *Variovorax*, *Azoarcus*, and *Anaeromyxobacter* sp. as well as the respiratory As(V)-reducing gene *arrA*.

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