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Interindividual variability of soil arsenic metabolism by human gut microbiota using SHIME model

Naiyi Yin [a, b], Huili Du [a, b], Pengfei Wang [a, b], Xiaolin Cai [a, b], Peng Chen [b], Guoxin Sun [b], Yanshan Cui [a, b, *]

[a] College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 101408, People's Republic of China
[b] Research Center for Eco-Environment Sciences, Chinese Academy of Sciences, Beijing 100085, People's Republic of China

HIGHLIGHTS

- Arsenic metabolism between adult and child was significantly different.
- Arsenic methylation ability of adult was 3-folds higher than that of child.
- Child gut microbiota exhibited high As(III) level with high health risk.
- Human gut microbiota from 20 various genera potentially had resistance genes.

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ABSTRACT

Arsenic (As) metabolism by human gut microbiota has been evidenced with *in vitro* experiments from contaminated soils. In this study, the variability in the metabolic potency toward As-contaminated soils and gut microbial diversity were investigated between healthy individuals (Adult versus Child). Arsenic bioaccessibility in the colon phase increased by 1.4–6.8 and 1.2–8.7 folds for adult and child, respectively. We found a high degree of As methylation for the colon digests of the adult (mean 2 μg methylarsenicals/hr/g biomass), 3-folds higher than that of the child. Besides, arsenite [As(III)] concentration (1.5–391.3 μg/L) for the child was 2–18 times for the adult. 16S rRNA gene sequencing revealed that human gut microbiota from 20 various genera potentially had resistance genes to reduce and methylate As under conservative statistics. Our results indicated that As metabolism by gut microbiota from adult and child was significantly different. The adult gut microbiota had a great ability of As methylation; the child gut microbiota exhibited high As(III) level, which could encounter high health risk. The identity and activity of arsenic-metabolizing bacteria isolated from human gut and its homologous role in As metabolism need be further explored. This study provides a better understanding of health risk assessment to adults and children upon soil As exposures.

1. Introduction

Arsenic (As) is ubiquitous as a toxic contaminant in natural environment and recognized as a class 1 carcinogen to humans (Wang and Mulligan, 2008). Soil is the main source of As exposure by inadvertent oral ingestion, especially through outdoor hand-to-mouth behavior by children (Ljung et al., 2006). Recently, *in vitro* methods with some advantages of ethical, economical, and time, have been recognized an appropriate surrogate to predict *in vivo* As relative bioavailability (RBA) (Bradham et al., 2011; Juhasz et al., 2014). There are numerous *in vitro* gastrointestinal methods estimating As bioaccessibility (the fraction of As that is soluble in the gastrointestinal environment of human being and available for absorption) in the gastric and small intestinal phases. The Simulator of Human Intestinal Microbial Ecosystem (SHIME) simulates a dynamic human gastrointestinal tract and cultures the colon microbial community of humans (Molly et al., 1994). The importance of As metabolism has been evidenced with *in vitro* experiments of human gut microbiota, which can induce As speciation changes and affect As bioaccessibility in contaminated media (Sun et al., 2012; Van de Wiele et al., 2010; Yin et al., 2015).

* Corresponding author. College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 101408, People's Republic of China.
E-mail address: cuiyanshan@ucas.ac.cn (Y. Cui).

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Generally, inorganic As (iAs) is the predominant form in the colon. The methylated As species-monomethylarsonic acid [MMA(V)], dimethylarsinic acid [DMA(V)], and highly toxic monomethylarsonous acid [MMA(III)] have been likewise detected (Van de Wiele et al., 2010; Yin et al., 2015; Yu et al., 2016). Rubin et al. (2014) described microbial thiolation leading to monomethylmonothioarsonic acid [MMMTA(V)]. In addition, recent studies on human urine analysis revealed some sulfur-containing methylated As metabolites, dimethylmonothioarsinic acid [DMMTA(V)], and dimethyldithioarsinic acid [DMDTA(V)] formed through As thiolation (Kubachka et al., 2009; Naranmandura et al., 2007). Nevertheless, human gut microbiota can actively volatilize As with the formation of highly toxic AsH_3 (arsine) (Michalke et al., 2008). In short, speciation analysis must be considered as an essential part of human health risk assessment, especially with respect to As toxicity largely determined by its species (Sun et al., 2014). Arsenic methylation was initially considered a detoxification process, but the formation of some methylated toxic As metabolites has forced researchers to reconsider the methylation process. Furthermore, As metabolism by human gut microbiota and intestinal absorption of these As metabolites should be concurrent processes. Intestinal absorption of different As standard species or metabolites is examined using *in vitro* studies with the Caco-2 human cell line of human colon carcinoma (Hinrichsen et al., 2015; Yin et al., 2017). In short, methylated trivalent arsenicals display a higher transport across epithelial cells in comparison with inorganic arsenic and the methylated pentavalent species. Besides, some thiolated methylarsenicals [DMMTA(V) and MMMTA(V)] are efficiently absorbed across Caco-2 cells.

The gut microbiota covers a large set of variable genes in numerous bacterial species with significant functions, which plays a number of important roles in human health. The colon represents a highly reducing environment, and harbors a vast (populated by a total of 10^{14} bacteria) and incredibly diverse microbial community, which reveals the metabolic potency toward xenobiotics (Schlebusch et al., 2015; Van de Wiele et al., 2010). However, the structure and activity of human gut microbiota from different individuals varies distinctly with diet, gender, and region (Arumugam et al., 2011; David et al., 2014; Greenblum et al., 2015); accordingly, variability in the diversity of human gut microbiota has been examined in populations with a wide range of ages. To date, limited efforts have been made to investigate differences between individuals regarding As metabolism by gut microbiota. When we conversely consider As exposure linked to a number of diseases, Lu et al. (2014) found that As exposure alters the gut microbial community at the abundance level, which substantially perturbs its metabolic profiles at the function level through the integrated metagenomics analysis.

In the present study, high performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS) was used to determine As metabolites, and to further evaluate variability in the metabolic potency toward soil As of gut microbiota from two healthy individuals (adult and child). Besides, we applied the 16S rRNA gene sequencing to characterize interindividual variability in gut microbial community. This study provides a better understanding of evaluating health risk to adults and children associated with soil As exposures, and can be as a reference to formulate related health risk policies.

2. Materials and methods

2.1. Arsenic-contaminated soils

Eight soils collected from a range of mining and farmland locations in China, covered a range of soil physicochemical properties and different concentrations of total As (Table 1). Air-dried soils were sieved to a particle size fraction of <250 μm that is most likely to stick to the hands of exposed humans. Soil pH was determined in water extracts (1:2.5 soil: deionized water) after 0.5 h of equilibration. Organic matter (OM) content was analyzed by UV/visible spectrophotometry after using acid dichromate oxidation method. A laser particle size analyser (Mastersizer, 2000; Malvern, U.K.) was used to obtain soil clay content (soil organic matter dislodged by H_2O_2). Amorphous iron and manganese oxides (Fe^{AO}, and Mn^{AO}) were extracted using 0.2 M acid ammonium oxalate. After microwave digestion (CEM MARS6) using a mixture of concentrated HNO_3, H_2O_2, and HF (5:3:2), total As and other metals concentrations were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Optima 7300V, PerkinElmer, U.S.) or inductively coupled plasma mass spectrometry (ICP-MS, 7500a, Agilent, U.S.). In the digestion process, blank sample and soil reference material GSS-5 (National Institute of Metrology, China) were included to ensure the internal quality assurance/quality control (QA/QC), whereas As recovery was 103.0± 3.2% ($n = 6$).

2.2. Dynamic SHIME

The *in vitro* colon microbial community utilized in the experiments was cultured and maintained in a modified SHIME, simulating the stomach, small intestine, ascending colon, transverse colon, and descending colon. Generally, fresh fecal microorganisms were obtained from a 28-year-old and a 6-year-old Chinese healthy male volunteer (no history of antibiotic treatment in the six months before this study), and separately inoculated into three colon compartments. Briefly, feed solution detailed in Yin et al. (2016), was added three times per day to provide digested nutrition for the colon microorganisms. After 3–4 weeks of adaptation, stable microbial communities were obtained in the colon microorganisms. The distal colon microbial fermentation activity (short chain fatty acid production and ammonium production) and community composition are investigated to be consistent with that of previous SHIME runs and an *in vivo* situation (Molly et al., 1994; Yin et al., 2015).

2.3. Arsenic metabolism of colon microbiota from contaminated soils

Arsenic bioaccessibility and its speciation in eight contaminated soils have been investigated through the combination of two *in vitro* methods, including the physiologically based extraction test (PBET) (Ruby et al., 1996) and the Unified Bioaccessibility Research Group of Europe (BARGE) method (UBM) (Wragg et al., 2011) with SHIME to subsequently simulate stomach, small intestine, and colon conditions, respectively. Briefly, soils (0.3 g) in triplicate were added to polypropylene conical centrifuge tubes (50 mL) with the gastric and small intestinal solutions (30 mL) at a soil/solution (s/s) ratio of 1:100. Following the small intestinal phase, these digests were transferred into 100 mL anaerobic serum bottles with 30 mL of colon solution from the descending colon compartment of the dynamic SHIME system at a double ratio (s/s) of the small intestinal phase. To obtain anaerobic conditions, the bottles were capped with butyl rubber stoppers and immediately flushed with nitrogen gas for 20–30 min. The digests were shaken at 150 rpm and incubated at 37 °C for 48 h.

All samples passed through 0.45 μm filter were subsequently stored at −80 °C until analysis. Arsenic concentrations in the small intestinal and colon (12 and 24 h) digests, were quantified using ICP-MS (diluted with 3% HNO_3), while the average recovery of check samples (20 μg L^{-1}) was 99.3% (94.5–100.6%, $n = 15$) during