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Biotransformation of arsenite and bacterial *aox* activity in drinking water produced from surface water of floating houses: Arsenic contamination in Cambodia

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ABSTRACT

The potential arsenite bioteansformation activity of arsenic was investigated by examining bacterial arsenic arsenite-oxidizing gene such as *aox*S, *aox*R, *aox*A, *aox*B, *aox*C, and *aox*D in high arsenic-contaminated drinking water produced from the surface water of floating houses. There is a biogeochemical cycle of activity involving arsenite oxidase *aox* system and the *ars* (arsenic resistance system) gene operon and *aox*R leader gene activity in *Alcaligenes faecalis* SRR-11 and *aox*S leader gene activity in *Achromobacter xylosoxidans* TSL-66. Batch experiments showed that SRR-11 and TSL-66 completely oxidized 1 mM of As (III) to As (V) within 35–40 h. The leaders of *aox*S and *aox*R are important for gene activity, and their effects in arsenic bioremediation and mobility in natural water has a significant ecological role because it allows arsenite oxidase in bacteria to control the biogeochemical cycle of arsenic-contaminated drinking water produced from surface water of floating houses.

1. Introduction

Arsenic is a ubiquitous environmental contaminant. It occurs in drinking water produced from surface water of floating houses, and in well water, ground water, with rice exposure, hair exposure, nail exposure, fish exposure, and in soil pollution. In Cambodia it continues to be a public health threat (Gault et al., 2008; Phan et al., 2014). Chronic arsenic exposure in Cambodia poses a health threat; it is present in due to human activities in rural and urban water flow due and causes illnesses due to unsafe water and/or unpurified drinking water (Phan et al., 2014; Sthiannopkao et al., 2010). Health problems associated with chronic exposure to arsenic-contaminated drinking water can include cancer, chronic arsenic poisoning, and the pathogenic potential of bacteria or fecal coliform, diabetes incidence, and blood stream infections (WHO, 2005). Cambodia is known as an endemic area for tuberculosis, intestinal parasites, malaria, measles virus, and *Escherichia coli* of fecal origin. The microorganisms present in water are involved in arsenic redox reactions in the natural state, and so may also be useful for detoxification, bioremediation, and metabolism.

Microbes can mediate the redox biotransformation of arsenic via arsenite oxidase (Chang et al., 2008). The biogeochemical cycle of arsenic has been brought about by the evolution of As-redox arsenic resistance system (*ars*) genes, encoding proteins that provide resistance through the oxidation of arsenite or reduction of arsenate by the bacterial strain (Yamamura and Amachi, 2014). Arsenite oxidase genes (*aox*S,-R,-A,-B,-C,-D) were found in *Alkalilimnicola ehrlichii strain* MLHE-1, *Alcaligenes faecalis*, *Chloroglexi* sp., *Marinobacteria* sp. SeaH-As6w, *Acinetobacter junii* SeaH-As6s, *Ochrobacterium tritici* SCII24, and α-, β-, and γ-Proteobacterium (Oremland et al., 2009; Paul et al., 2015; Guo et al., 2015). Arsenic detoxification and metabolism processes suggested that *aox*S-*aox*R are the lead genes, and mediate regulatory control of *aox*AB, and the transformation potential of As species. Thus, arsenite oxidase is important in the biogeochemical cycle of As species change. Oremland et al. (2005) showed that a biogeochemical arsenic cycle is operative in the sediments of salt-saturated extreme environments and in Mono Lake in California. However, basic research is also needed on how microbial processes affect arsenic and detecting microbial communities in less extreme environments, such as drinking water, groundwater, or aquifer systems (Das et al., 2013). Considering the background of this paper, arsenic contamination in groundwater can enter surface water in various ways to cause damage to humans; this is especially the case for indigenous

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floating-house residents who obtain drinking water from wells (pump) as well as surface water. While the Cambodian government is dedicating resources to remove water-based arsenic toxicity, a biologically feasible solution comprises an evaluation of the toxicity-reduction features of arsenite-oxidase genes, whereby the arsenic-resistance systems of the Earth's microorganisms is used.

The objectives of the present study were (i) to assess arsenic contamination compared with the extent of health risk assessment with respect to different levels of As-rich drinking water, surface water, red tube well water, drinking water, ground water, rice exposure, nail exposure, hair exposure, fish exposure, and soil exposure in Cambodian environments, (ii) to determine the potential pathogenicity of stream microorganisms and how their ecology affects human activities in Cambodia, and (iii) to assess the arsenic detoxification potential of arsenite-oxidizing bacteria isolated from drinking water produced from surface water of floating houses. The *aox*-mediated transformation is proposed to have an important influence on the As biogeochemical cyclic activity in the Siemreap River and Tonleap Lake in Cambodia.

2. Methods and methods

2.1. Sampling prearation and analyses

Surface water was collected from 10 sites of arsenic-contaminated drinking water for the floating houses (Siemreap River: latitude 13° 11′ N, longitude 103° 49′ E and Tonlesap Lake: latitude 12° 38′ N, longitude 103° 52′ E) in Cambodia and the unpolluted control areas (Yeongsan River: latitude 34° 47′ N, longitude 126° 28′ E and Damyang Lake: latitude 35° 17′ N, longitude 127° 0′ E) in the Republic of Korea in February 2006 (Fig. 1). Surface water samples were filtered through a membrane filter (Whatman 0.45 μm) and stored at 4 °C until the analysis. The temperature, pH, turbidity (NTU), and conductivity of the drinking water samples were measured in the field using an Orion electrode Orion model 290A portable meter fitted with an Orion model 9107 electrode, and biochemical oxygen demand (BOD_5; 5-day BOD by standard method, (Jouanneau et al., 2014)) and chemical oxygen demand (COD; EPA method, 410.1). All drinking water samples were transported to the laboratory on ice and then stored at 4 °C until analyzed. Samples of drinking water were separated into two portions, and then filtered through a 0.45 μm filter (Whatman 0.45 μm pore size, 13 mm), after nitric acid was added to give a pH of ~2. The arsenic concentration was measured using inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500). *E. coli* were grown in 100 mL cultures of MI medium (Difco, USA) and incubated at 35 ± 2 °C for 6–8 h, according to EPA method supplementary data from one colony forming unit (CFU/100 mL) (USEPA, 2002). All analytical measurements were performed in duplicate.

2.2. Pure culture condition of arsenite-oxidizing bacteria

All the surface water samples(100 μL) were innoculated into the Stainer's basal medium (MSB) supplemented with 1 mM sodium arsenite, $NaAsO_2$ (Sigma) or sodium arsenate ($Na_2HAs(V)O_4$) (Aldrich, St. Louis, MO, USA). After several transfers, the isolated colonies were evaluated to determine if they contained arsenite, and each isolate were then further assessed to determine if it was aerobic or anaerobic. The sodium arsenite (III) was used at a concentration of 26 mM by the 7th pure culture. After several transfers, isolated colonies were assessed for arsenic(III), and every single isolate was selected. Arsenic resistance experiments were carried out using a variety of arsenic concentrations (As(III): $NaAsO_2$ at 0, 5, 10, 15, 20, and 26 mM) for all the isolates. All arsenite-oxidizing bacteria were cultured at 28 °C in MSB medium (pH 7.0) that contained 1 mM D(+)-glucose as the carbon source. Anaerobic pure cultures that were isolated using the arsenic resistance system (MSB) media were then placed in an anaerobic chamber (Coy Laboratory, MI, USA) and incubated at 28 °C (gas mixture: 5% CO_2, 10% H_2, and 85% N_2).

2.3. Genomic DNA isolation

Bacterial genomic DNAs were prepared from the bacterial cultures using standard methods (Sambrook and Russel, 2006), and were placed in a 1-mL microcentrifuge tube for each individual colony. Bacterial suspensions were composed of 10^7 CFU/mL of each isolate. Each culture was incubated overnight at 28 °C with intermittent shaking. The culture was then placed in a 1.5-mL microcentrifuge tube with 1 mL of TES (10 mM Tris–HCl, 50 mM EDTA, 10% sodium dodecyl sulfate) and 10 μL of proteinase K (50 mg L^{-1}), which was reacted in a 55 °C shaking waterbath for 10–12 h to digest the protein. An equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added to the reaction mixture. This solution was then manually mixed for 3 min and centrifuged (14,240 × g, 15 min) and the supernatant was removed. This process was repeated three times. The supernatant was then mixed with 0.1 volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of 100% ethanol. Tubes were shaken slowly and the genomic DNAs were gently removed and then cleaned with 75% ethanol, dried, and dissolved in TE (10 mM Tris pH 8.0, 1 mM EDTA pH 7.2). The DNA purity was measured using a spectrophotometer at 260 nm and 280 nm. All DNA had an absorbance ratio (A_{260}/A_{280}) ranging from 1.7 to 2.0.

2.4. PCR amplication of 16S rRNA gene and aox genes and phylogenetic analysis

The sequences of primer sets used for PCR of 16S rDNA and the *aox* genes are presented in Supplemental Table S1. Genomic DNA was isolated from pure-culture bacteria. The PCR amplification of 16S rDNA gene was performed with the universal primer set: BGRI-09 sense (5′-ATC ATG GCT CAG ATT GAA CGC -3′) and BGRI-15 antisense (5′-T ACC TTG TTA CGA CTT CTA CCT-3′) primers. The large fragment of the 16S rRNA gene was ~1.5 kb in size. The amount of genomic DNA of the arsenic resistant bacteria added to the PCR mixture was ~50 ng in the final volume. The PCR for each gene amplification consisted of 5 min of pre-denaturation at 95 °C, 35 cycles of 1 min denaturation at 94 °C, 1 min annealing at 55 °C and 1.5 min extension at 72 °C, and a final cycle of extnsion at 72 °C for 7 min. The PCR was conducted in a Mastercycler Gradient thermalcycler (Eppendorf, Germany), and the PCR products were identified by gel electrophoresis using 0.7–1.2% agarose gels. The samples were then analyzed using an automated DNA sequencer (Model 3100; ABI PRISM Genetic DNA Analyzer System Profile, South Korea). The 16S rRNA gene sequences were compared with the NCBI database using the BLAST algorithm integrated with the Vector NTI Suite (ver. 5.5.1; InforMax, USA) system (Altschul et al., 1997). Database sequences with fewer than 1200 ~ 1500 nucleotides were excluded from the phylogenetic analysis, and the almost-complete 16S rRNA gene sequence of the isolated bacterium was aligned with closely related sequences retrieved from GenBank using CLUSTAL_X (15). Phylogenetic analysis was performed using the neighbor-joining method (Thompson et al., 1997).

2.5. Redox assays of arsenite-oxidizing bacteria

Batch tests were performed independently in triplicate using 250-mL glass flasks that contained 60 mL of MSB medium, 1 mM sodium arsenite $NaAsO_2$ (Sigma) and 1 mM D(+)-glucose. For the batch tests, the drinking water-isolated bacteria (10^7/CFU) were