Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jes

JES JOURNAL OF ENVIRONMENTAL SCIENCES

www.jesc.ac.cn

Arsenic redox transformation by *Pseudomonas* sp. HN-2 isolated from arsenic-contaminated soil in Hunan, China

*Zhennan Zhang, Naiyi Yin, Xiaolin Cai, Zhenzhou Wang, Yanshan Cui**

1. *College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 101408, China. E-mail: zhangzhennan10@mails.ucas.ac.cn*
2. *Research Center for Eco-environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China*

ARTICLE INFO

Article history:
Received 17 September 2015
Revised 12 October 2015
Accepted 4 November 2015
Available online 8 April 2016

Keywords:
Arsenic
Arsenite oxidation
Arsenate reduction
Detoxification
Pseudomonas

ABSTRACT

A mesophilic, Gram-negative, arsenite[As(III)]-oxidizing and arsenate[As(V)]-reducing bacterial strain, *Pseudomonas* sp. HN-2, was isolated from an As-contaminated soil. Phylogenetic analysis based on 16S rRNA gene sequencing indicated that the strain was closely related to *Pseudomonas stutzeri*. Under aerobic conditions, this strain oxidized 92.0% (61.4 μmol/L) of arsenite to arsenate within 3 hr of incubation. Reduction of As(V) to As(III) occurred in anoxic conditions. *Pseudomonas* sp. HN-2 is among the first soil bacteria shown to be capable of both aerobic As(III) oxidation and anoxic As(V) reduction. The strain, as an efficient As(III) oxidizer and As(V) reducer in *Pseudomonas*, has the potential to impact arsenic mobility in both anoxic and aerobic environments, and has potential application in As remediation processes.

Introduction

Arsenic(As) is a natural and ubiquitous metalloid that ranks the 20th most abundant in the earth's crust (Cullen and Reimer, 1989). Many people around the world are exposed to As-polluted underground water and soil and suffer from chronic As poisoning, which causes hyperkeratosis, cancer, diabetes, cardiovascular disease, and other diseases (Sumi and Himeno, 2012).

Arsenic occurs in several oxidation states including arsenate (As(V)), arsenite (As(III)), elemental As (As0) and arsenide (As(−III)). Arsenic is mostly found in inorganic forms as trivalent arsenite (As(III)) or pentavalent arsenate (As(V)) (Cullen and Reimer, 1989). Among them, As(III) is generally regarded as more mobile and more toxic than As(V) (Liu et al., 2001). The affinity of As(III) for protein thiols or vicinal sulfhydryl groups makes it highly toxic. As(III) also acts as an endocrine disruptor by binding to hormone receptors and interfering with normal cell signaling (Kaltreider et al., 2001). As(V) is a chemical analog of phosphate with subsequent inhibition of oxidative phosphorylation (Goyer and Clarkson, 1996).

The contribution made by microorganisms to the biogeochemistry of As in the environment is extensive and detailed. Microbial activity strongly influences As speciation. Various bacteria are known for their ability to transform inorganic As species through oxidation or reduction processes (Jones et al., 2012; Oremland and Stolz, 2003). For chemoautotrophic As(III) oxidizers, the oxidation of As(III) generates energy, which promotes their growth and proliferation (Santini et al., 2000); whereas for heterotrophic As(III) oxidizers, the oxidation of As(III) is described as a detoxification mechanism catalyzed

* *Corresponding author*. E-mails: zhangzhennan10@mails.ucas.ac.cn (Zhennan Zhang), cuiyanshan@ucas.ac.cn (Yanshan Cui).

http://dx.doi.org/10.1016/j.jes.2015.11.036

by the periplasmic enzyme arsenite oxidase (Muller et al., 2003). Dissimilatory arsenate-reducing prokaryotes conserve energy by linking the oxidation of lactate to the reduction of As(V) to As(III). Reduction of As is also carried out by resistant microbes utilizing an As(V)-reductase and As(III)-expulsion pump (Tsai et al., 2009). Biotic arsenic transformation could cause either arsenic release or immobilization in soil. It was found that bacterial activity was responsible for the reduction of arsenic, causing the solubilization of the metalloid from pyrite cinders to the aqueous phase (Corsini et al., 2010). Oxidation of As(III) to As(V) contributes to improved immobilization of As and thus helps to mitigate As contamination (Sun et al., 2010).

In order to better understand the mechanism of biotic As transformation in the environment and further understand the combined effect of abiotic (*e.g.*, mineral oxidants and humus) and biotic factors on As transformation in soil, isolation of As-transformation bacteria is necessary. Furthermore, the microbial transformation of As(III) to As(V) might represent an eco-friendly, cost-effective alternative approach to conventional remediation processes (Bahar et al., 2012). Several bacterial strains have been isolated and identified as As(III) oxidizers since the first arsenite-oxidizing bacterium was described in 1918 (Green, 1918), including *Stenotrophomonas* (Bahar et al., 2012), *Polaromonas* (Osborne et al., 2010), *Bacillus* (Shivaji et al., 2005), *Alcaligenes* (Osborne and Ehrlich, 1976), *Agarobacterium* (Salmassi et al., 2002), *Thiomonas* (Bruneel et al., 2003), *Microbacterium lacticum* (Mokashi and Paknikar, 2002) and *Bosea* (Liao et al., 2011). Dissimilatory reduction of As(V) has been shown to occur in at least nine different genera scattered throughout the domain Bacteria and has also been observed in two hyperthermophilic Archaea (Macur et al., 2004). The As(V)-reducing bacteria utilizing a detoxification or resistance mechanism have also been identified and classified as *Pseudomonas*, *Psychrobacter*, *Vibrio*, *Citrobacter*, *Enterobacter*, *Bacillus* (Liao et al., 2011), *Alcaligenes* (Cavalca et al., 2013), *Pantoea* (Wu et al., 2013), *Agrobacterium*, *Flavobacterium* and *Microbacterium* (Macur et al., 2004). These bacteria have been generally isolated from soils (Bahar et al., 2012; Majumder et al., 2013), groundwater (Liao et al., 2011; Mokashi and Paknikar, 2002), Hot Creek (Salmassi et al., 2002), mines (Santini et al., 2000), the Arctic Circle (Osborne et al., 2010), sediments (Valenzuela et al., 2009) and plant effluents (Bachate et al., 2013). Although various kinds of As-transformation bacteria have been found, the expression by a single organism of both reductive and oxidative metabolic functions, has, to our knowledge, only been observed so far in members of *Thermus*, *Bacillus*, *Marinobacter*, *Comamonas*, *Flavobacterium*, *Staphylococcus*, and *Pseudomonas* genera (Fisher and Hollibaugh, 2008; Gihring and Banfield, 2001; Handley et al., 2009). These bacteria mentioned above were isolated from hot springs, lakes, marine hydrothermal sediments and plants. No soil bacterial strain with both As(III)- oxidizing and As(V)-reducing ability has been reported before.

In this article, we presented (i) the isolation of a strain with the ability of both heterotrophic As(III)-oxidation and As(V)-reduction from an As-contaminated soil, (ii) phylogenetic analysis of the strain, (iii) the identification and characterization of arsenite oxidase and arsenate reductase genes, (iv)As oxidation and reduction assays.

1. Materials and methods

1.1. Isolation and growth of As-resistant bacteria

A soil sample ([As] = 284 mg/kg) collected from a mine in Zhuzhou, Hunan, China, was used as an inoculum for enrichment culture. The isolation and subsequent growth experiments were carried out with a modified chemically defined medium (CDM) (8.12 mmol/L $MgSO_4$, 18.7 mmol/L NH_4Cl, 7 mmol/L Na_2SO_4, 0.0574 mmol/L K_2HPO_4, 0.457 mmol/L $CaCl_2$, 44.6 mmol/L Na-lactate and 9.5 mmol/L $NaHCO_3$, with the pH adjusted to 7.0) as described by Weeger et al. (1999). As-resistant bacteria were enriched with 1.33 mmol/L As(III) under oxic incubation. 1 g soil samples were placed in sterile flasks containing CDM supplemented with 1.33 mmol/L As(III), and incubated at 25°C on a rotary shaker (170 r/min) in the dark for 48 hr. After incubation, samples were serially diluted, and a cell suspension was spread onto CDM plates with 1.33 mmol/L As(III) at 25°C for 48 hr to obtain single colonies. A pure culture was obtained by successive isolation of colonies at 25°C in As(III)-supplemented medium.

1.2. Selection of as(III)-oxidizing strain

The ability of the obtained As-resistant bacteria to oxidize As(III) was tested using a qualitative $AgNO_3$ screening method as described by Liao et al. (2011). Briefly, exponential-phase culture was centrifuged and then resuspended in CDM containing 1 mmol/L As(III). The bacterial suspensions were incubated at 30°C for 72 hr. Subsequently, the bacterial cultures were centrifuged, and 100 μL of the liquid phase was mixed with 100 μL of 0.1 mol/L $AgNO_3$ solution. The resulting precipitates containing As showed colors ranging from the light yellow of Ag_3AsO_3 (silver orthoarsenite) due to As(III) to the light brown-red of Ag_3AsO_4 (silver orthoarsenate) due to As(V). The observation of a light brown-red precipitate was regarded as indicating an As(III)-oxidizing strain. After confirmation of a pure As(III)-oxidizing strain, bacteria were preserved in 30% glycerol at −80°C.

1.3. Bacterial As(III) oxidation experiment

The ability of the bacterial isolate to oxidize As(III) was further quantitatively determined by batch tests. The cells were grown in CDM. Once the cells reached the exponential phase, they were harvested by centrifugation at 5000 r/min for 20 min at 4°C and cell pellets were resuspended in the required volume of CDM to obtain a cell density of about 10^7 CFU/mL. The test medium was supplemented with 5 mg/L (66.7 μmol/L) of As(III) and incubated on a rotary shaker (150 r/min) at 30°C. The pH value of the medium was adjusted to 7.0. Controls without inoculation were also incubated under the same conditions. Samples were taken over time for measurement of cell density and for determination of As speciation. Samples were centrifuged, decanted and stored at −20°C prior to As analyses. Dissolved oxygen (DO), oxidation reduction potential (ORP) and pH were also monitored. All experiments were done in triplicate, and the mean values were taken into account.