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Research article

Isolation and identification of the native population bacteria for bioremediation of high levels of arsenic from water resources

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ABSTRACT

Health of millions of people is threatened by the risk of drinking arsenic-contaminated water worldwide. Arsenic naturally conflicts with the concept of life, but recent studies showed that some microorganisms use toxic minerals as the source of energy. Hence, the researchers should consider the development of cost-effective and highly productive procedures to remove arsenic. The current study was conducted on a native bacterial population of Seyed-Jalaleddin Spring Kurdistan, Iran. Accordingly, the arsenic amount in water samples was measured >500 μg/L by the two field and in vitro methods. Water samples were transferred to laboratory and cultured on chemically defined medium (CDM) with arsenic salts. A total of 14 native arsenic-resistant bacterial strains were isolated and after providing pure culture and performing biochemical tests, the isolates were identified using polymerase chain reaction (PCR) and 16s rRNA genomic sequencing. The potential of bacterial strains for the biotransformation of arsenic was assessed by the qualitative assessment of $AgNO_3$ method and efficiency of arsenic speciation was determined for the first time by silver diethyldithiocarbamate (SDDC) method with an error of less than 5%. Among the isolated strains, only strain As-11 and strain As-12 showed arsenic transformation characteristics and were registered in NCBI database by the access numbers KY119262 and KY119261, respectively. Results of the current study indicated that strain As-11 had the potential of biotransformation of As(V) to As(III) and vice versa with the efficiency of 78% and 48%, respectively. On the other hand, strain As-12 had the potential for biotransformation of As(V) to As(III) and vice versa with the efficiency of 28% and 45%, respectively.

1. Introduction

Arsenic is a rare metal ranked the 20th regarding its frequency in the earth crust (Jolly, 1966), and is found combined with igneous and sedimentary rocks (Merian et al., 2004). Many springs and water running resources are naturally contaminated with arsenic. The regular consumption of such water resources by human beings gradually causes diseases such as melanosis, gangrene, cancer, and finally, death (Hopenhayn, 2006; Maleki et al., 2015). Geothermal areas usually generate springs with high concentration of arsenic (Webster and Nordstrom, 2003). Concentrations more than 50 μg/L of arsenic in drinking water resources increases the risk of bladder and lung cancers and total amounts less than 50 μg/L in drinking water may cause skin cancer. Hence, arsenic in water is a high risk for human health (Jebelli et al., 2017; Lizama et al., 2011). Since the toxic effects of arsenic results from its oxidation state (Pongratz, 1998), identification of all arsenic-resistant strains and the evaluation of factors influencing the speciation are important issues to remove arsenic from the environment. The most frequent forms of

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arsenic in the environment are its inorganic compound known as arsenite As(III), followed by arsenate As(V) (Balasoiu et al., 2001). The toxicity, bioavailability, and mobility of arsenic in the environment are highly associated with its binding capacity (Bissen and Frimmel, 2003; Inskeep et al., 2001). The mobility of As(III) is more than that of As(V) and accordingly, it is more toxic (Mahimairaja et al., 2005). The main factors controlling the speciation of arsenic are the oxidation state and pH (Masscheleyn et al., 1991).

Arsenic remediation process from drinking water was introduced by the United States Environmental Protection Agency (EPA) includes sedimentation, absorption, ion exchange, and membrane processes. In addition to rather fine removal efficiency for the above mentioned processes (80%–95%), they also had disadvantages such as high cost of administration, high efficiency just in small scales, necessity for secondary refining in most of the cases, sludge production with the high concentration of arsenic, sludge disposal, water loss 10%–15% (based on water quality and the employed process known as a main problem in low-water areas), and the selective performance of some processes during the exposure to different ions concentration (Pirnie, 1999). Most of such techniques are used to remove As(V) and are not efficient enough to remove As(III). Hence, a pre-oxidation stage is required to transform As(III) to As(V) (Katsoyiannis et al., 2004). As(III) oxidation is usually done by adding chemical compounds such as ozone, oxygen peroxide, chlorine, or potassium permanganate (Jekel, 1994; Kim and Nriagu, 2000). Although such compounds affect the oxidation of As(III), due to the formation of hazardous byproducts resulted from their consumption, the costs of coping with the byproducts dramatically increases. In the recent years, biological remediation of arsenic was introduced as a cost-effective method. Hence, microbial oxidation of As(III), as a suitable alternative for the chemical oxidation, can be employed (Duarte et al., 2009).

Microorganisms play important roles in the bioenvironmental state of arsenic, by different effective mechanisms to transform soluble to insoluble species of arsenic as well as toxic to nontoxic ones (Jebeli et al., 2017). There are 4 different microbial mechanisms in the transformation of arsenic as methylation, demethylation, oxidation, and reduction (Gihring et al., 2001; Ilyaletdinov and Abdrashitova, 1981; Oremland et al., 2000; Ridley et al., 1977; Sohrin et al., 1998; Stolz and Oremland, 1999). Therefore, in order to determine the efficiency of arsenic microbial oxidation, various analyses on different species of arsenic in biological samples are required. In recent years, different methods have been introduced to measure the concentration of arsenic species. However, there is still no common method of measurement in this subject. (Bednar et al., 2004; Matera et al., 2003; Rasmussen et al., 2002). The current study aimed at 1) determining the native arsenic-resistant population of bacteria in Emamzadeh Seyed-Jalaleddin travertine spring water, 2) identifying the bioprocess of the spring and 3) measuring the concentrations of arsenic species in the biological samples for the first time by the in vitro method of SDDC, based on the accuracy and validity of the tests, availability, and ease of use. SDDC is a spectrophotometric-based method; it benefits from the possibility to measure different species of arsenic separately and without the need for expensive instruments.

2. Materials and methods

2.1. Site description and sampling

Samples were collected in 4 steps from April to October 2014 from the arsenic-contaminated Emamzadeh Seyed-Jalaleddin Spring, 18 km from Ghorveh city, Kurdistan province, Iran. The geographic coordinates of the spring were between 35°17′22″N latitude and 47°54′14″E longitude. Water samples were collected in the specific 1-L polyethylene bottles washed previously with nitric acid 5% and double distilled water. Samples were stored at 4 °C and transferred to laboratory after adding 1 mL of concentrated nitric acid. Temperature, pH, and electrical conductivity (EC) were measured by a portable Orion multiparameter device and the total amount of arsenic was measured using the Quantofix field kit at sampling site. Then, samples were collected from water and sediments of the spring in the sterile bottles and immediately transferred to laboratory at 4 °C for the microbial analyses.

2.2. Arsenic measuring in water samples

Total arsenic was measured by SDDC method and, then controlled by the inductively coupled plasma-atomic emission spectrometry (ICP-AES) method. Most of the developing countries determine the arsenic concentration in water by the standard method of SDDC, which is extensively used due to its ease of use (American Public Health et al., 2010). All of the chemicals used in this method were reagent grade, and they were used without further purification. In this method, As(III) optionally reduces to arsine (AsH_3) under aquatic conditions by sodium borohydride ($NaBH_4$ 37.83M - pH 6). The produced arsine gas was removed by the oxygen-free nitrogen gas and then, after passing through a reduction column filled with glass wool or cotton impregnated with lead acetate solution [$Pb(CH_3COO)_2 \cdot 3H_2O$] (to prevent hydrogen sulfide intervention), entered SDDC absorbing solution and morpholine solved in chloroform. Red color intensity was measured at 520 nm wave length by a spectrophotometer, which indicated As(III). To measure the total amount of inorganic arsenic in the absence of methylarsenic compound, the sample was reduced to pH 1. Alternatively, arsenate is measured in a sample from which arsenite has been removed by reduction to arsine gas at pH 6 as above. First of all, the sample was acidized using hydrochloric acid 2M and, then, sodium borohydride was added. Arsine, composed of As(V), entered SDDC absorbing solution and morpholine was solved in chloroform. Red color intensity was measured at 520 nm wave length by a spectrophotometer, which indicated As(V). To draw absorption curve, the water synthetic samples with the total volume of 70 mL containing 0, 1, 2, 5, 10, 15, and 20 μg were prepared; then, the arsenic remediation was conducted and the absorption curve was drawn based on the absorption against chloroform and the obtained absorption equation (Fig. 3). The accuracy and validity of the analyzing method was determined in water samples contaminated with different concentrations of arsenic (50, 250, 500, and 1000 μg).

2.3. Isolation and identification of arsenic-resistant bacteria

To isolate arsenic-resistant bacteria, chemically defined medium (CDM) medium, as described previously by Weeger et al., was used (Weeger et al., 1999). For this purpose, a 10-mL of water sample was inoculated to 100 mL of CDM broth containing 2 mM of As(III) or 10 mM of As(V) and the bacterial cultures were placed on a rotary shaker and incubated under aerobic conditions at 25 °C. Then, every 48 h subcultures were prepared on CDM agar containing 2 mM As(III) or 10 mM of As(V) and to obtain single colonies, the plates were incubated at 25 °C for 10 days.

To isolate bacteria from sediments, 100 g of sediments was mixed with 50 mg of sodium arsenite ($NaAsO_2$) and, then, incubated at 25 °C for 1 week. Next, 1 g (dried weight) of the mixture was added to 9 mL of 0.85% NaCl and shaken for 10 min. Serial dilutions were prepared from the provided solvent and, then, cultured on CDM agar containing 2 mM As(III); the spread plate method was used to obtain single colonies. The plates were incubated at 25 °C.