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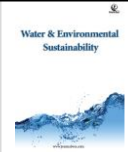

Research paper

Isolation and Biochemical Characterization of Arsenic resistant Bacteria from Textile Effluent Contaminated Soil of Jaipur, Rajasthan

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A R T I C l E I N F O

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A B S T R A C T

Arsenic occurs naturally in the environment, but its uncontrolled liberation from industrial effluents has been imposing adverse effects on the environment. The continuous exposure of the soil is a matter of concern in this study. The soil consortium contains bacterial colonies that resist and adapt the metal toxicants and can in turn help in the bioremediation of such metals from the soil. This study stresses the isolation of arsenic resistant bacteria from the arsenic-contaminated soil. The Soil Sample was collected from the Sanganer area of Jaipur, Rajasthan. The soil in this area received the textile discharge from the industries located nearby. Four arsenic resistant bacteria were isolated from the sample which showed a high tolerance level towards arsenic and was able to grow in the presence of arsenic in-vitro. The Minimum inhibitory concentration was also determined for the strains against arsenite. The highest MIC was found to be 16mM of arsenite, which concludes tolerable limits of the strains. The Biochemical and Morphological characterization of the isolates was also conducted. The Four isolates also showed resistance towards various other metals like cadmium, cobalt, lead, zinc, mercury, chromium, and tin. The isolates on biochemical characterization were found to belong to the following Genus: Moraxella, Azomonas, Acetobacter and Corynebacterium. This resistance capacity of the isolates depicts their potential to bioremediate the toxicity of the arsenic in the environment.

Introduction

Arsenic is the twentieth abundant element on earth's crust, and the most toxic element encountered by the environment. It is present in both organic as well as inorganic forms, where inorganic forms of arsenic are more toxic than organic forms (Hopenhayn, 2006). Arsenic enters the terrestrial and aquatic environment, as the result of both geogenic processes and anthropogenic activities. Geogenic sources include natural weathering of rocks, burning vegetation, volatilization and volcanic action, mining, soil forming biogeochemical processes etc. While Anthropogenic sources include combustion of municipal waste, arsenic pesticides (herbicides, fungicides, insecticides), land filling of industrial wastes, release or disposal of chemical warfare agents, petroleum refining , pharmaceutical manufacturing etc. (Derome, 2000, Deshpande and Pande, 2005, Ewers, 1991). Arsenic cannot be destroyed once it enters the environment (Valls and De Lorenzo, 2002) which poses harmful effects on humans, animals, plants and other organisms. Most commonly found inorganic forms include, arsenate [As(V)] and arsenite [As(III)]. Arsenite is 100 times more toxic and mobile than arsenate (Cullen and Reimer, 1989, Nakamuro and Sayato, 1981, Neff, 1997). The adverse effects of exposure to these inorganic forms can cause decrease in production of RBC and WBC, the significant amount can also result in developing cancer associated with skin, lungs, bladder and kidney, liver and lymphatic system (Hopenhayn, 2006).

There are different methods for mitigating arsenic from environment like watershed management, community participation and bioremediation strategies. But, bioremediation is considered to be a more efficient, cost effective, eco friendly method for the removal of arsenic in affected areas (Dabrowska et al., 2012, Das et al., 2009, Michel et al., 2007, Tsai et al., 2012). Bioremediation involving microorganisms plays a vital role in environmental remodeling. There are various mechanisms by which microbes can overcome arsenic toxicity. The mechanisms providing microbes resistance are chiefly based on minimizing the amount of arsenic that can enter the cell. These methods include extracellular precipitation, exclusion of metal ions, intracellular sequestration, catalyze transformations of arsenic including oxidation, reduction, and methylation etc. These strategies are used by bacterial cells either for detoxification or for energy generation to support cellular growth (Cullen and Reimer, 1989, Dowdle et al., 1996, Newman et al., 1998, Satchanska et al., 2005). The research conducted by different scientist have also reported to isolate various species of strict aerobic As (III)-oxidizing and facultative anaerobic As (V)-reducing bacteria from As-contaminated sites (Abbas et al., 2018, Suhadolnik et al., 2017) which suggest both kind of bacteria have potential to resist arsenic forms.

This study aims to isolate and characterize the bacterial strains having potential to tolerate arsenic in the environment. It will help to explore the bioremediation capacity of the microbes to detoxify the contaminant in the soil receiving textile discharge in Sanganer, Jaipur.

Material and Method

1. Isolation of Arsenite resistant bacteria from contaminated soil

The soil sample was collected from the land near the drain in the month of January. The sterile polypropylene zip lock bags were used to store the samples. The soil was sampled approximately from the depth of 10-15cm. The presence of arsenic and its concentration in the soil was estimated by Atomic Absorption Spectroscopy (AAS, model Chemito AA2013).

The bacterial strains were isolated from soil samples on nutrient broth supplemented with the required concentration of Sodium arsenite and pure culture was obtained by repeated streaking, spreading on nutrient agar plates. Gram staining was also performed for preliminary identification.

2. Estimation of Minimum Inhibitory Concentration (MIC) of isolates against arsenic

Isolated pure colonies were inoculated in nutrient broth medium supplemented with increasing doses of sodium arsenite (mM) to determine the lowest concentration, which completely inhibits bacterial growth. This concentration at which the growth ceases is considered as minimum Inhibitory concentration of the toxicant for the isolate. (Satchanska et al., 2005). It was determined by optical density of the culture at 600nm (UV-Vis Spectrophotometer) on the basis of negligible growth.

3. Biochemical Characterization of the isolates

Biochemical properties of the strains were tested according to Bergey's Manual of Systematic Bacteriology, while procedures of these tests were taken from Cappuccino and Sherman (Cappuccino and Sherman, 2005). Biochemical tests included utilization of various sugars, H_2S production, IMVIC test, citrate utilization, Oxidase, Catalase activity, Starch hydrolysis, nitrate reduction and urease activity. These tests resulted in the determination of genus identification which could be the type of strain isolated which will be confirmed by 16s rDNA sequencing in later studies.

4. Determination of other metal resistance in strains

Circular discs made by Whatman No 1filter paper were soaked overnight in each metal solution containing 100µg/l concentration. These discs were then oven dried and placed on the nutrient agar plates. Resistance towards metals was inferred by the Zone of inhibition around the discs which were observed after 48 hours of incubation. The metals used for testing included cadmium, cobalt, lead, nickel, zinc, mercury, chromium and tin salts. The presence of arsenic and its concentration was estimated by Atomic Absorption Spectroscopy (AAS, model Chemito AA2013).

Results and Discussion

1. Isolation of Bacterial strains

High concentration of arsenic i.e 423 ppm in the soil was estimated using Atomic Absorption Spectroscopy (AAS, model Chemito AA2013). The presence of arsenic in the soil can be supported by the work of researchers like Jaishree and Khan *et al.*, 2014, Mahawar *et al.*, 2015 as they suggest the textile effluents discharged after processing of product contains heavy metals.

Four bacterial isolates were obtained from the soil samples of Sanganer, jaipur. These isolates were assigned Lab nomenclature as- AE-1, AE-2, AE-3 and AE-4. Gram staining showed AE-1 as gram negative cocci while AE-2,AE-3 as gram negative bacilli and AE-4 as Gram positive Bacilli. Gram staining explained the preliminary characterization of strains.

2. Determination of Minimum inhibitory concentration (MIC) of isolates against arsenic

The purified four bacterial isolates showed resistance towards arsenic with MIC from 7mM to 16mM. The strains AE-1, AE-2, AE-3 and AE-4 exhibited MIC of 7.7mM, 13.9mM, 11.6mM and 16mM respectively. The dose at which the strains showed complete growth inhibition was analysed by optical density taken at 600nm. The MIC for respective strains can be observed in Figure1.

Among the bioremediation strategies Microorganism plays a key role in biogeochemical cycling of heavy metals. They use various mechanisms like efflux of metal ions outside the cell, accumulation, complexation of metal ions inside the cell, oxidation of metal ions to less toxic forms , this helps bacteria to tolerate the metal in stress conditions (Nies, 2000).

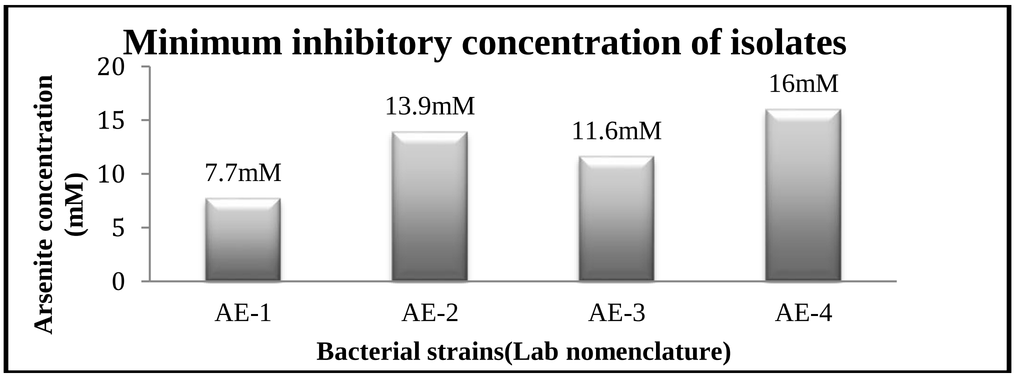

Figure 1. The Minimum Inhibitory Concentration of the four isolates from contaminated soil sample

3. Biochemical Characterization of the isolates:

Table 1. Biochemical results of isolated strain and expected genus identification

Biochemical Tests	AE-1	AE-2	AE-3	AE-4
Gram Staining	-ve	-ve	-ve	+ve
Dextrose fermentation	-ve	+ve	+ve	+ve
Lactose fermentation	-ve	+ve	+ve	-ve
Sucrose fermentation	-ve	+ve	+ve	+ve
H_2S production	-ve	-ve	-ve	-ve
Indole test	-ve	-ve	-ve	-ve
Methyl Red test	+ve	+ve	+ve	+ve
Voges-Proskauer test	-ve	-ve	-ve	-ve
Citrate test	-ve	-ve	-ve	+ve
Catalase activity test	+ve	+ve	-ve	+ve
Oxidase test	+ve	+ve	-ve	-ve
Starch hydrolysis	+ve	+ve	-ve	-ve
Nitrate reduction	+ve	+ve	-ve	+ve
Urease activity	+ve	+ve	+ve	+ve
Genus identification	*Moraxella,*	*Azomonas,*	*Acetobactor*	*Corynebacterium*

+ve=Positive, -ve= negative

All the strains were tested for biochemical procedures to identify the Genus. Results of different biochemical tests are summarized in Table1. On the basis of biochemically characterization the strains were identified using Bergey's Manual of Determinative Bacteriology which suggested AE-1, AE-2, AE-3 and AE-4 could be from Genus *Moraxella, Azomonas, Acetobactor* and *Corynebacterium* respectively.

Three strains i.e. AE-1, AE-2, AE-3 were found to be Gram negative, while AE-4 was Gram positive. The result shows the potential of both gram positive and negative strain having potential of resisting arsenite. The studies conducted by (Bachate et al., 2009, Ghodsi et al., 2011, Titah et al., 2018) have also reported the potential of both gram positive and gram negative bacterial isolates to show arsenic resistance. Basha et al in 2014 also isolated bacteria from the textile contaminated soil containing heavy metals in Chennai, and the resistance towards these metals was also reported.

4. Determination of other metal resistance in the strains:

Resistance to the metals were recorded as positive if the growth occurred around the metal discs whereas the clear zone without the growth around the metal discs were considered as negative or a "zone of inhibition". Results after 48 hours suggested resistance towards all the metals (Table2) by four strains to some extent, except the greater zone of inhibition of 11mm and 16mm was observed in AE1 and AE-2 respectively for Nickel. This concludes high sensitivity towards nickel by the two strains. According to Durve *et al.*, 2012, microbes possess various mechanisms to combat the toxic effect of heavy metals. The isolated microbes (*Pseudomonas aeruginosa* and *Brevibacillus choshinensis*) from industrial effluent discharges were able to tolerate high levels of cadmium, lead, arsenic and mercury.

Table 2. Multi metal resistance exhibited by isolated bacterial strains

Metals(100µg/ml)	AE-1	AE-2	AE-3	AE-4
	Zone of inhibition and Susceptibility	Zone of inhibition and Susceptibility	Zone of inhibition and Susceptibility	Zone of inhibition and Susceptibility
Cadmium Chloride ($CdCl_2.5H_2O$)	No Zone/Resistant	No Zone/Resistant	No Zone/Resistant	No Zone/Resistant
Cobalt Nitrate ($Co(No_3)_2.6H_2O$)	No Zone/Resistant	No Zone/Resistant	No Zone/Resistant	No Zone/Resistant
Lead Nitrate ($Pb(NO_3)_2$)	No Zone/Resistant	No Zone/Resistant	>10mm	No Zone/Resistant
Nickel Chloride ($NiCl_2.6H_2O$)	11mm	16mm	No Zone/Resistant	No Zone/Resistant
Zinc Sulphate ($ZnSO_4.H_2O$)	No Zone/Resistant	Slightly Sensitive	No Zone/Resistant	No Zone/Resistant
Chromium Chloride ($CrCl_3.6H_2O$)	No Zone/Resistant	No Zone/Resistant	No Zone/Resistant	No Zone/Resistant
Sodium Selenate (Na_2SeO_4)	No Zone/Resistant	No Zone/Resistant	No Zone/Resistant	No Zone/Resistant
Stannous Chloride ($SnCl_2.2H_2O$)	No Zone/Resistant	No Zone/Resistant	No Zone/Resistant	No Zone/Resistant
Antimony Chloride ($SbCl_3$)	>10mm	Slightly Sensitive	No Zone/Resistant	No Zone/Resistant
Mercuric Chloride ($HgCl_2$)	No Zone/Resistant	No Zone/Resistant	No Zone/Resistant	No Zone/Resistant

Conclusion and Significance:

Degradation of soil due to industrialization and frequent liberation of wastewater runoff directly into the environment has become an alarming problem. This deterioration of quality of soil is not only causing ecological risk but also affecting social and economic issues. The presence of heavy metal toxicants like arsenic is released in textile effluent can be witnessed in this study conducted on the soil of Sanganer area of Jaipur, Rajasthan. Natural restoration is done by nature regularly, but when human intervention leads to excessive discharge of chemicals then this natural process fails.

In this study four isolates were obtained which biochemically and morphologically characterized to belong to genus *Moraxella, Azomonas, Acetobactor* and *Corynebacterium* respectively. All the four isolates showed resistance to almost all metals taken in this study and were also able to grow in presence of sodium arsenite showing maximum MIC of 16mM. The high resistance levels of the microbes towards arsenite make them beneficial for bioremediation strategies in contaminated sites. This resistance ability can be advantageous to mankind and can also help in developing eco friendly technology to treat textile effluent affected soil and water ecosystems. Further studies on these bacterial strains and on their genes is needed to understand the mechanism, which in turn will help in enhancing the arsenic bioremediation process.

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