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Assessment of Arsenic Biosorption Kinetics of Isolated Indigenous Multi-Heavy Metal Tolerant Bacteria

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Abstract

The intrinsic properties of heavy metals, such as arsenic, chromium, and lead, as well as their widespread dispersion, make them a significant environmental hazard. Arsenic is specifically categorized as a carcinogen of group 1. Arsenic contaminated soil and water is hazardous because it penetrates into the food chain and causes health problems. The present study aimed to investigate the bioremediation potential of arsenic-resistant bacteria. An exhaustive investigation was undertaken to isolate 26 bacterial strains exhibiting resistance to heavy metals. The ability of these isolated strains to assimilate arsenic from water solutions containing both As III and As V forms was then evaluated. Nineteen isolates demonstrated varying degrees of efficacy in the absorption of arsenic. Among these, the F1 isolate had shown the capability to uptake 30 µg/L As III and 32 µg/L As V The growth patterns of F1 indicated that the control group exhibited greater expansion than the group that received arsenic. Due to bioremediation potential of arsenic-resistant isolates, these isolates could help to bioremediate and restore As-contaminated environments. This research offers valuable insights into the mechanisms and kinetics of arsenic biosorption, thereby facilitating the advancement of environmental friendly and economically viable bioremediation approaches to mitigate arsenic pollution in soil and water.

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Introduction

The widespread distribution and toxicity of heavy metals including chromium (Cr), arsenic (As), and lead (Pb) have made them a major environmental issue (Hasan *et al*., 2016; Maarof *et al*., 2016; Aldawsari *et al*., 2017). One well-known carcinogen is As, which is classified as a category 1 carcinogen (WHO 2011). Its impacts on human and environmental health are incredibly harmful and poisonous (Bhakta *et al*., 2016). Among the many serious health problems that arsenic may cause are hyperpigmentation, skin cancer, kidney damage, liver cancer, circulatory irregularities, unfavorable pregnancy outcomes, neurotoxicity, and arsencosis (Quansah *et al*., 2015). Based on the aforementioned perspectives, it is evident that water and soil pollution with arsenic poses a significant and dangerous threat to human health. This is because a considerable number of individuals worldwide are exposed to arsenic through the food they consume, making it a silent killer. The remediation of polluted water and soil poses significant difficulties because of the nonbiodegradable nature of As, which persists in the environment for an extended period (Naushad *et al*., 2015; Maarof *et al*., 2016). To mitigate this, several scientists have developed

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various methods for purifying contaminated water, such as sorbent media, various filters, treatment devices, and more (Bhakta *et al*., 2016; Bhakta and Ali, 2020). Regrettably, these technologies are expensive, impractical, malfunctioning in real-world applications, lacking user-friendliness, and unaffordable for the general populace. For instance, filters, absorbent mediums, treatment devices, and other similar methods have been found to have low efficiency in removing As and lack technical support after being installed in real-world settings. Because of this, the amount of arsenic in the treated water starts to rise sharply not long after the device is set up (Bhakta *et al*., 2016). The use of various microorganisms to extract heavy metals from water solutions has recently emerged as an exciting eco-technology. Heavy metals include As, Cd, Pb, and others. Bhakta *et al*. (2012a, b) discovered some lactic acid bacteria (LAB), specifically Enterococcus faecium and Lactobacillus reuteri, that are resistant to cadmium and lead. These bacteria can be used to remove cadmium and lead from water. Although the majority of living things are killed by arsenic, certain bacteria may live in areas where groundwater contains high amounts of arsenic. These bacteria significantly influence arsenic's geochemical behaviour (Duan *et al*., 2009). Consequently, getting microbes that can both accumulate and resist arsenic is the first step toward addressing the concern of arsenic-contaminated groundwater. According to Pepi *et al*. (2007), several bacteria, including Aeromonas, Bacillus, and Pseudomonas, have significant resistance to both As (III) and As (V).

Based on the study discussion, it is evident that there is a lack of sufficient study on the biosorption capacity of As-resistant microbial isolates. The goals of this study were to determine the kinetics of As bioremediation, the growth patterns of microbial isolates, and the isolation of multi-heavy metal tolerant bacteria that are resistant to and capable of uptaking As.

Materials and Methods

Sample collection and bacterial isolation

Bacterial strains resistant to arsenic were recovered from soil and tannery effluent samples acquired at several locations in Bangladesh, including Faridpur, Hemayetpur, and Kalicharanpur (Table 1). Isolating bacteria that are resistant to arsenic required diluting the rhizospheric soil thoroughly before 50 µL of water samples were put onto nutrient agar plates that had 10 mM concentrations of As(III) and As(V) (Shakoori *et al.*, 2010). Bacterial proliferation was detected following a 24 hour incubation period at temperature of 37 °C. After that, isolates of colonies were collected and streaked many times on a nutritional agar medium (HiMedia, India) that had 10 mM arsenic, in the form of As(III) and As(V), respectively. While a pure culture was prepared, the chosen isolates were streaked onto nutrient agar media that had different quantities of As added to it. After that, the mixture was incubated at 37°C for 24 hours or until the lowest concentration growth was achieved. The concentration of the sample was calculated using the procedures outlined by Dos Santos *et al.* (2018).

Preparation of heavy metals (As, Cr and Pb) solution

Arsenic trioxide (As_2O_3, As_2O_5,, Cica-Reagent, Kanto Chemical Co., Inc., Tokyo, Japan) had to be dissolved in Milli Q (MQ) water in order to create a stock solution of arsenic (As) with a concentration of 2000 mg/lL. In accordance with the procedure outlined by Bhakta *et al*. (2010, 2014), the mixture was autoclave sterilized and kept at 4 °C in a sealed glass container. By employing this stock solution, we prepared solutions with varying amounts of As for the subsequent experiments.

Actinobacterial isolates were subjected to metal resistance using chromium (Cr) and lead (Pb), both classified as heavy metals. Ingredients of the highest quality were utilized in the preparation of the salt solutions, specifically $K_2Cr_2O_7$ and $PbNO_3$. Separate sterilisation procedures were carried out on each solution, with a temperature of 110°C applied for 15 minutes each (Saurav and Kannabiran, 2009). Using stock solutions of 1,000 mg/L, the metal ion concentrations were generated.

Screening of potential as uptaking bacterial isolates

The most efficient isolates for aspartate absorption were identified by assessing their capacity to

remove aspartate from water, utilizing an adjusted method based on the approach outlined by Bhakta *et al*. (2012a). Following three washes in 50 ml falcon tubes with MQ water, the cells that had been collected from the 18-hour fresh culture were centrifuged at 8000 g for 10 minutes.After being resuspended in sterile As solutions (0.5 mg/L), the cell pellet from the isolates was cultured at 37°C. The pellet had a wet weight of 100 mg/ml. Two milliliter eppendorf tubes were used to collect the water samples following a fourteen-hour incubation period. This was followed by 10 minutes of centrifugation at 8000 g for the tubes. Accurately transferring one milliliter of water from the supernatant, a Shimadzu ICPS-1000IV equipment based on inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used to assess the quantity of total arsenic. The metal-removal efficiency (MRE) equation, as proposed by Bhakta *et al*. (2012b), was employed to calculate the isolate's capability to remove arsenic.

The equation follows: C_i and C_f indicate the initial and final concentrations of As in water, M signifies a cell's mass, while t_f and t_i are the predefined final and initial timings, respectively. The MRE values in this investigation were expressed in milligrams per hour per gram (mg/h/g).

Determination of growth pattern of potential as uptaking isolates

The freshly developed LAB strains were anaerobically incubated overnight with shaking (150 excrursions/ min) at 37 °C. The LAB strains were then injected in triplicate into broth mediums. The control medium contained simply MRS (De man, rogosa and sharpe agar), whereas the treatment medium contained MRS supplemented with 5 mg/L of As. As supplementation was used at a dosage of 5 mg/L to measure the optical densities (ODs) of the control group, whereas the treatment group used MRS broths without supplementation at 550 nm.

Determination of comparative growth and growth pattern under heavy metals stress

To assess the level of heavy metal resistance in specific microbial isolates, un-inoculated broth was used as a control, and the optical density (OD) was assessed at 550 nm. The broth contained lead nitrate $Pb(NO_3)_2$ and potassium dichromate $K_2Cr_2O_7$ at concentrations of 0.5, 0.75, 2, 4, 6, 8, 10, and 12 mM. The cultures were inoculated into a liquid medium and subsequently exposed to varying concentrations (0, 0.50, 0.75, 2, 4, 6, 8, 10, and 12 mM) of $Pb(NO_3)_2$ and $K_2Cr_2O_7$. The samples were then incubated at 37^0C temperature to observe the growth behaviour of the isolates.

Kinetics of arsenic bioremediation

The optimal influencing factors for eliminating As from the ambient aqueous phase were determined by conducting as bioremediation kinetic experiments on a chosen isolate. The studies were conducted using a pre-existing stock solution and measuring the moist weight of recently cultured and triple-washed isolates. To determine the total arsenate content in the water that remained after centrifugation of the experimental samples, we used an Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES) model ICPS-1000IV made by Shimadzu in Tokyo. It was determined that the chosen strain of isolates had a high As biosorption capability by applying the formulas given by Bhakta *et al.* (2012b).

The variables in the equation are defined as follows: qe denotes the sorption capacity of As in milligrams per gram (mg/g); Ci and Ce indicate the initial and final concentrations of As in milligrams per litre (mg/L). Similarly, V denotes the volume of the As solution in litres (L), and M represents the bacterial isolates' weight in grams.

Statistical analysis

Each experiment's data was subjected to statistical analysis with three experiment repetitions. The statistical analysis package SPSS 10 (SPSS Inc, USA) was used to determine the mean values and the least significant difference.

Results and Discussion

Isolation of multi-heavy metal tolerant bacteria

There were a total of twenty-six microorganisms that were tolerant to a number of different heavy metals (F1-11, S 1-4, R 1-7, K 1-3, and RJ), were isolated from various sources (Faridpur, Hemayetpur, and

Kalicharanpur) and varying concentrations of $PbNO_3$ and K_2CrO_4. As shown in Table 1, most of the isolates had shown significant growth at the concentrations of $PbNO_3$ (2 mM, 4 mM, and 6 mM) and K_2CrO_4 (0.50 mM and 0.75 mM), while subsequent increase of $PbNO_3$ concentrations doses exhibited unfavourable growth patterns. The findings suggest that faecal samples serve as a reservoir of bacteria that can tolerate multiple heavy metals. These bacteria can potentially be utilized in the biotechnological industry for advantageous applications.

Table 1. Growth of isolates at different concentration of $PbNO_3$ and K_2CrO_4

Isolate	Sample source	$PbNO_3$						K_2CrO_4	
		2 mM	4 mM	6 mM	8 mM	10 mM	12 mM	0.50 mM	0.75 mM
F1	Faridpur	+	+	+	+	–	–	+	+
F2		+	+	+	+	–	–	+	+
F3		+	+	+	–	–	–	+	+
F4		+	+	+	+	–	–	+	+
F5		+	+	+	–	–	–	+	+
F6		+	+	+	–	–	–	+	+
F7		+	+	+	–	–	–	+	+
F8		+	+	+	–	–	–	+	+
F9		+	+	+	+	–	–	+	+
F10		+	+	+	–	–	–	+	+
F11		+	+	+	–	–	–	+	+
S1		+	+	+	–	–	–	+	+
S2	Hemayetpur	+	+	+	+	+	+	+	+
S3		+	+	+	–	–	–	+	+
S4		+	+	+	+	+	+	+	+
R1		+	+	+	–	–	–	+	+
R2		+	+	+	–	–	–	+	+
R3		+	+	–	–	–	–	+	+
R4		+	+	+	+	+	+	+	+
R5		+	+	+	–	–	–	+	+
R6	Kalicharanpur	+	+	+	+	+	+	+	+
R7		+	+	+	–	+	–	+	+
K1		+	+	+	–	+	–	+	+
K2		+	+	+	–	–	–	+	+
K3		+	+	+	+	+	+	+	+
RJ		+	+	+	+	+	+	+	+

"+" = Grown, "–" = Not grow

Screening of potential as uptaking bacteria

The bacteria, which can tolerate many heavy metals, were able to remove arsenic from the water. Among 26 multi heavy metal resistance bacterial isolates, 19 isolates had shown As assimilation capability at variable concentration of As supplements which has been shown in Figures 1 and 2. When supplemented with As (III) (sodium arsenite) at a concentration of 50mM, the arsenic concentration in the water ranged from 1 to 31 µg/L (Figure 1). Similarly, when the water was supplied with As-V at a dosage of 250 mM, the arsenic content in the water varied between 1 and 32 µg/L (Figure 2). The results clearly revealed that the 19 isolated bacterial strains with tolerance to arsenic have varying efficiency in absorbing arsenic. Among 19 As uptaking bacterial isolates, the S1 and F1 isolates exhibited maximum As uptake (31 µg/L for S1 and 32 µg/L for F1) capability which was isolated from a solution containing sodium arsenite (50 mM). The variation in arsenic removal values obtained by the selected isolates indicates that each isolate may belong to distinct As-resistant LAB species and/or strains (Bhakta *et al*., 2022).

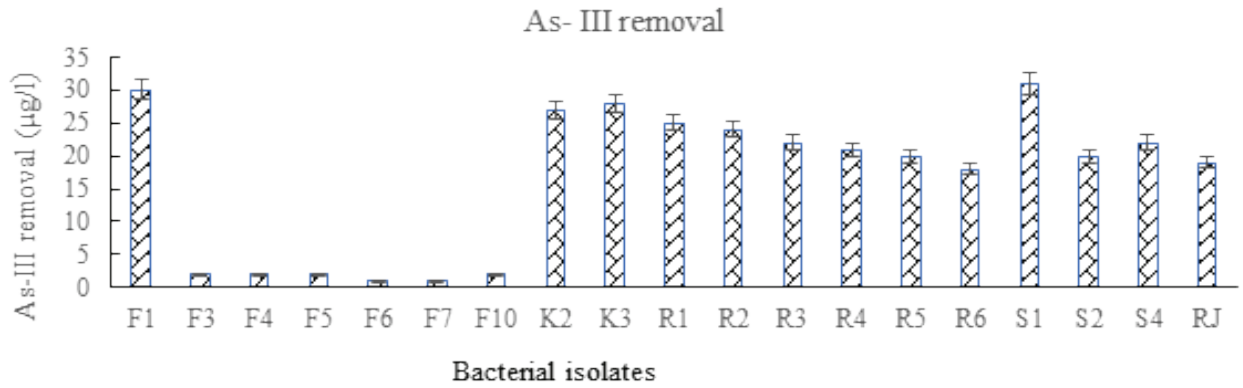

Figure 1. Arsenic removal level of multi-heavy metal tolerant bacteria supplemented As (III) (sodium arsenite) (50 mM) aqueous phase

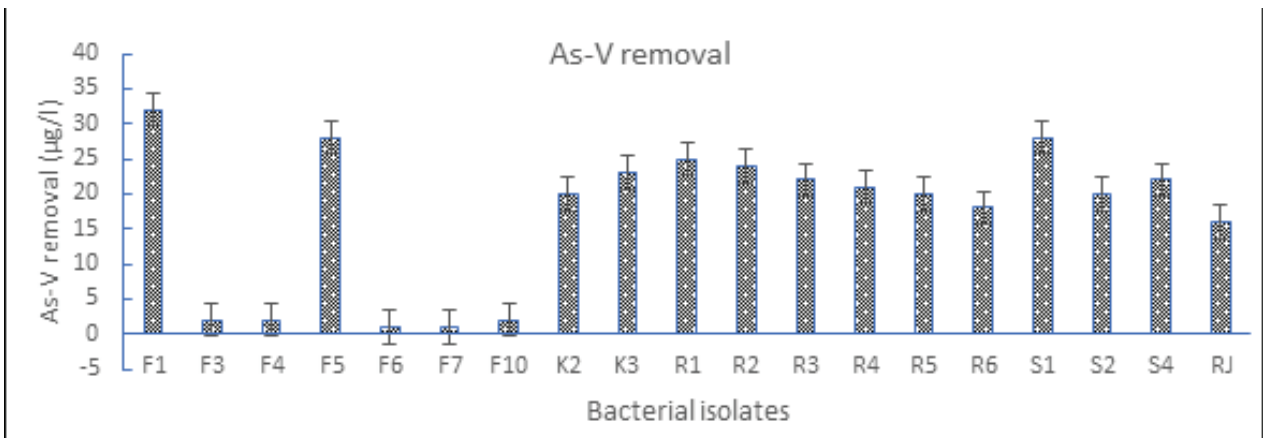

Figure 2. Arsenic removal level of multi-heavy metal tolerant bacteria supplemented As (V) (250 mM) aqueous phase

The metagenomic mud and sludge samples were collected by Bhakta et al. (2010) and they found several LAB that could reduce arsenic (As). Finding lactic acid bacteria (LAB) in environmental samples that can withstand heavy metals has been the subject of several research (Ameen *et al.*, 2020; Huët *et al.*, 2017; Bhakta *et al.*, 2012a).

Growth Pattern of Potential As Uptaking Multi Heavy Metal Tolerant Bacteria

The control group's growth varied from 1 to 10 optical density (OD) points, whereas the treatment group's growth ranged from 1 to 7.5 OD points (Figure 3). Following 18 hours of growth, the control group's bacterial isolate F1 reached a maximum of 10 optical density (OD) while the As treatment group reached 7.5 OD (Figure 3). During the lag, log, and stationary phases of the growth cycle, the As-treated bacterial strain F1 showed considerably decreased growth. As treatment resulted in a 20% decrease in average growth rate for bacterial isolate F1 when compared to the control group. F1 exhibited the most significant growth inhibition, reaching 27%, during the log phase's culmination at 18 hours, representing the maximal growth phase. Nevertheless, the suppression of As growth is contingent on the concentration. Lead ions inhibited the development of Lactobacillus L. plantarum YW11 in the latter logarithmic or early stationary phases, according to Liu *et al.* (2019). The LAB isolate-HS12, when treated as intended, exhibited significantly reduced growth during many phases of the growth cycle, including the lag, log, and stationary phases (Bhakta *et al.*, 2022).

Arsenic bioremediation kinetics

Effect of aqueous phase

Figures 4a and 4b illustrate how the bioremediation kinetics of As are affected by the aqueous phase of multi-heavy metal-tolerant bacteria. When treated with As (III) (50 mM) and As (V) (250 mM) aqueous phases, the kinetics of arsenic bioremediation of multi-heavy metal-resistant bacteria exhibited distinct trends.

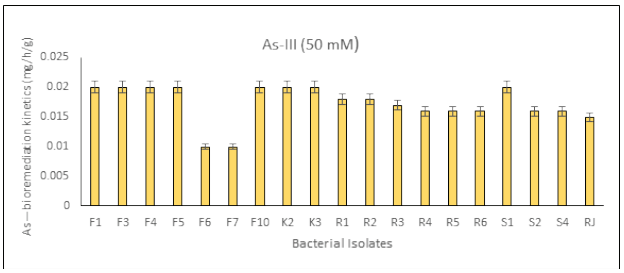

Figure 4a. Arsenic (As) bioremediation kinetics of multi-heavy metal tolerant bacteria supplemented As III (50 mM) aqueous phase

The highest As bioremediation kinetics were determined to be 0.02 mg/h/g in the aqueous phase supplemented with As (III) (50 mM), whereas the aqueous phase supplied with As (V) (250 mM) was found to be 0.023 mg/h/g. According to Khan *et al.* (2016), while dosage negatively impacts biosorption capacity, total Cd biosorption in *Salmonella enterica* 43^0C is increased. Similar results on the impact of biomass concentration on metal biosorption ability were discovered by Al-Garni (2007).

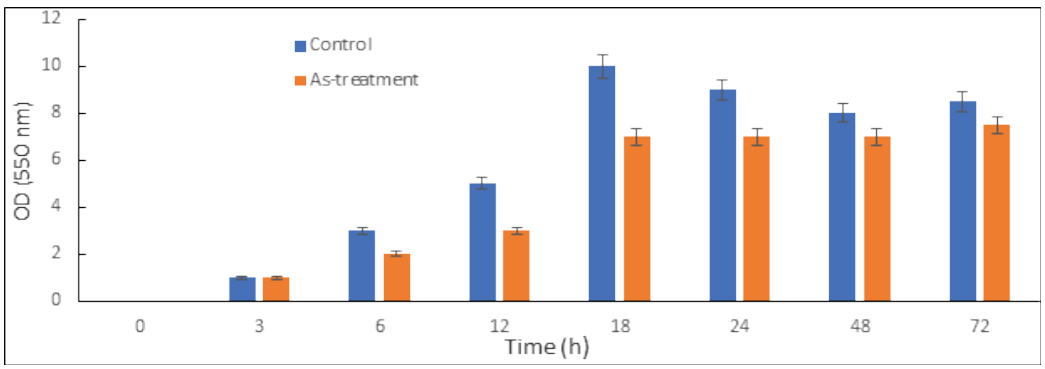

Figure 3. Growth pattern of selected As-tolerant bacterial isolate F1 showing growth inhibition of F1 in As-supplemented MRS medium compared to that of the control one.

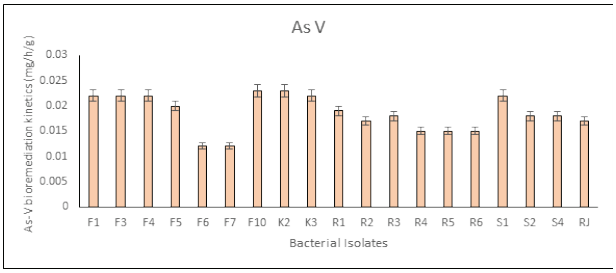

Figure 4b. Arsenic (As) bioremediation kinetics of multi-heavy metal tolerant bacteria supplemented with As (V) (250 mM) aqueous phase.

Conclusions

The increasing environmental peril of heavy metals, particularly arsenic, requires immediate and effective remediation techniques. The findings of this study revealed that specific bacteria with tolerance to multiple heavy metals showed notable resistance to arsenic. The isolates exhibited diverse levels of effectiveness in removing arsenic, highlighting their potentiality for use in bioremediation process. Arsenic exposure significantly impacted the growth patterns of the chosen isolate (F1), resulting in substantial decreases in growth at various stages of the growth cycle. This suggests the possible suppressive impact of arsenic on bacterial proliferation. In addition, the study examined the rate at which the selected organism absorbs substances from its environment, specifically focusing on its ability to remove arsenic. This investigation yielded valuable information on the optimal factors that affect the efficiency of arsenic removal. Altering environmental factors to increase arsenic biosorption efficiency should be the primary goal of future studies.

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