Study on *Burkholderia sp*: Arsenic Resistant Bacteria Isolated from Contaminated Soil

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Abstract

The threat of arsenic (As) pollution being severe warrants opting for low cost microbial remediation strategies. Our present study of identifying suitable bacterial strain led to isolation of As-tolerant strain from the As contaminated soil of West Bengal, India. The isolated bacterial strain LAR-2 had a high MIC (minimum inhibitory concentration) towards As(V) (300.67 mM) and As(III) (31.3 mM) and transformed 78.4% of arsenite to arsenate under laboratory condition. Based on 16S rRNA homology the LAR-2 was identified as *Burkholderia sp* (MK634685) and emerged as the most potent strain for As decontamination.

Keywords: Arsenic (As), *Burkholderia sp*, minimum inhibitory concentration, phylogenetic tree

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1. Introduction

The eastern India (West Bengal mainly) and Bangladesh are severe sufferers of arsenic contamination owing to exposure of living system via water and food [1,2]. The use of arsenic or heavy metal enriched water in agricultural fields is the reason of its significant build-up in soil [3,4] and subsequent accumulation in standing crop [5]. As residues are generally found in the top layer of soil or surface soil and enter the plants easily because of its low volatility and low solubility [6]. As has both organic and inorganic forms [7] and in environment found as an oxyanion [8]. Several soil detoxification methods such as stabilization, containment, and solidification etc. have been offered for the in-situ remediation of contaminated soils, but the high-risk involvement and high cost of remediation [9] emphasizes the scope of bioremediation through novel As-tolerant microbes [10]. Bioremediation through novel As-tolerant microbes may be an easy, eco-friendly, low cost and emerging approach to mitigate arsenic contamination. Soil microorganisms can volatilize arsenic from the soil [13,14]. *Alcaligenes faecalis*, *Bordetella* sp. and *Rhodobacter sphaeroides* are three most important arsenic volatilizing bacteria also previously reported.

In this backdrop, we have categorized the study and identified efficient As-resistant bacterium from contaminated soil, and accrue steps to contribute an accelerated remediation of arsenic polluted soils.

2. Materials and Methods

2.1. Collection and Analysis of Soil Samples

Soil samples (2 cm diameter, 10 cm depth) were collected aseptically from arsenic (As) contaminated rhizospheric zone of lentil in Chakdaha, West Bengal (23°05’ N latitude and 88°54’ E longitude), India, noted for As concentrations in the ground water above World Health Organization (WHO)-defined safe limit [15]. Atomic Absorption Spectrophotometer (AAS) was used to measure the total [16] and available As [17] of the soil samples.

2.2. Isolation of As-tolerant Bacteria

2 g of soil (taken from lentil rhizosphere separately) was suspended in 2 mL sterile distilled water, 1mL of each was re-suspended in Yeast Extract Mannitol (YEM) liquid medium separately in two conical flasks, spiked
with 1 mM As(III) and 1 mM As(V). The total experimental set up was incubated for 48 hours at 30°C [18]. After incubation 2 ml culture was further inoculated in a YEM liquid medium. Procedure was repeated twice. Around 0.1 mL of As spiked culture was spread on a YEMA (Yeast Extract Mannitol Agar) solid medium plate to isolate the As tolerant bacteria.

2.3. MIC (Minimum Inhibitory Concentration) Value of the Bacteria

The MIC is the lowest concentration of As(V) or As(III) which entirely inhibits microbial growth and activity [19,20]. 1.0 mL of overnight bacterial culture was inoculated in two conical flasks containing 99.0 mL of YEM liquid medium, containing either As(III) (NaAsO₂; 1-50 mM) or As(V) (Na₂HAsO₄·7H₂O; 1-500 mM) separately and incubated at 30°C with 48 hrs of shaking. The OD (optical density, measurement of microbial growth) of the bacterial cultures was detected using a UV-Vis spectrophotometer at 600 nm wavelength.

2.4. Oxidation and Reduction of Arsenic by the Isolates

Standard silver nitrate (AgNO₃) method was employed to screen As-oxidizing bacterial isolates [21]. The Arsenic-oxidizing ability of the bacterial strains was further confirmed using a modified micro plate technique [22].

2.5. Arsenite Oxidase Assay

The arsenic tolerant bacterial isolates were grown in CDM medium spiked with 30 mM of arsenite. After centrifugation at 10000 rpm for 2 minutes late log-phase cells were collected. Then cells were washed by using 50 mM Tris-HCl buffer (pH 8.0) and suspended in 2 mL buffer having 0.5 mM phenyl methyl sulfonyl fluoride (PMSF) and lysozyme. Cell suspensions were chronosuccessfully incubated for 2 h and sonicated for disrupting the cellular membrane. Then after centrifugation at 10,000 rpm for 30 min cell debris was removed. Finally, the protein concentrations were measured by using bovine serum albumin (Sigma) as a standard [23]. The arsenite oxidase assay was performed using a method previously described [24].

2.6. Identification of the As Tolerant Bacteria

The As tolerant bacterial genomic DNA was isolated and PCR amplification for molecular identification by 16S rRNA gene sequence analysis was done [25]. The bacterial isolate was studied for Gram reaction, colony morphology and characterized for catalase, urease, and oxidase activities by standard protocols [26].

2.7. Scanning Electron Microscopic (SEM) Study

For the SEM study the bacterial cells were harvested first and then washed with sodium phosphate buffer (pH 7.4). A thin bacterial smear was prepared on a cover glass, heat fixed over a flame for 1-2 sec followed by fixation with 2.5% glutaraldehyde for 45 min [27]. The slides were then dehydrated with 50-90% of alcohol solutions and finally through absolute alcohol for 5 min each and observed under a 15 kV scanning electron microscope (HITACHI, S-530, SEM and ELKO Engineering).

2.8. Statistical Analysis

Statistical computations were performed using Microsoft Excel 2016 and SPSS version 23.0.

3. Results

3.1. Characterization of Experimental Site

The total As concentration and available As concentration of experimental rhizospheric soil of lentil were measured. Results revealed a considerable load of the total and available arsenic i.e. 17.2 ± 1.72 and 1.50 ± 0.27 mg kg⁻¹ respectively (presented as mean of three observations ± SD).

3.2. Isolation and Identification of the As Tolerant Bacteria

A few distinct colonies were found in the YEMA medium (Figure 1). From these a distinct colony was picked and allowed for further study.

![Figure 1. Burkholderia sp. in congo red YEMA medium](image)

The isolated strain of Burkholderia was found to be gram negative and rod shaped. This isolated strain was studied for phenotypic and biochemical studies and have been presented in Table 1. The bacterial isolate was tested for catalase, urease, citrate, oxidase, indole, MR, VP.

| Biochemical Tests | Performance |
|------------------|-------------|
| Catalase         | +           |
| Oxidase          | -           |
| Urease           | +           |
| Indole           | -           |
| Citrate utilization | +        |
| Methyl Red(MR)   | -           |
| Voges-Proskauer (VP) | -       |

Further employing 16S rRNA gene sequencing a phylogenetic tree was prepared (Figure 2). These identified bacterial isolates thus assumed to be Burkholderia sp (LAR-2, accession number MK634685).
Further to confirm the results, the SEM study of the bacterial isolates (Figure 3) was also categorized.

### 3.3. MIC of the Arsenic Resistant Bacterial Isolates

The arsenic tolerant strain was tested for their minimum inhibitory concentration of As. The result showed that LAR-2 (*Burkholderia sp.*) had a high MIC value (Table 2). Bacterial isolate had emerged with a high MIC towards As(V) (300.67 mM) and As(III) (31.3 mM). Further to establish the As oxidizing potential, silver nitrate test was executed (Figure 4), which also provided the rate of As(III) oxidation by the strain as 988 μM h⁻¹ and capacity of arsenite oxidation to the tune of 78.4%.

### Table 2. Minimum Inhibitory Concentration and arsenite oxidase Enzyme activity of the arsenic tolerant bacteria

| Name of the Bacteria | Arsenate (V) (mM) | Arsenite(III) (mM) | Enzyme activity (nM min⁻¹ mg⁻¹ protein) | Rate of As(III) Oxidation (μM h⁻¹) | Percentage of arsenite oxidation (%) |
|----------------------|------------------|-------------------|----------------------------------------|-----------------------------------|-------------------------------------|
| LAR-2                | 300.67±5.00      | 31.3±1.00         | 4.97±0.6                               | 988                               | 78.4                                |
| Control              | 0                | 0.1               | 0                                      | -                                 | -                                   |

### 4. Discussion

#### 4.1. Identification and Isolation of As-resistant Bacterial Strains from Contaminated Soils

The As pollution in the contaminated experimental zone is geogenic. Soil pH, redox condition (Eh) and organic matter, which also effect on soil microbial populations, governs the degree of arsenic contamination and relative proportions of arsenic species [28]. The arsenic resistant bacteria are diverse and the prolonged contamination induced native microbial communities establish a surviving power to exist under metal stress condition [29]. In our study, the soil microclimate was predominated by heterotrophs [30]. The bulk of the As-resistant microbes were isolated from ground water [31], sediments [32], soil [33] etc. Diverse groups of bacteria including *Bordetella* [30], *Achromobacter* [34],
was found to oxidise 1250 μM h−1 [35]. The isolate, in the present investigation, were observed to show resistance to arsenite (up to 31.3 mM) and arsenate (up to 300.67 mM) which were significantly higher than As-oxidising bacteria isolated from ground water [36], soil [37] and estuaries [38].

The isolates under the present investigation, LAR-2 were identified as Burkholderia (based on 16S rRNA gene sequence homology) which can oxidize 988 μM h−1 (78.4 %) presence of an arsenite concentrations (30 mM) within 24 h under laboratory condition. Bacillus megaterium was found to oxidise 1250 μM h−1 of As(III) (100%) in the presence 30 mM As(III) in the culture media under laboratory conditions [25].

5. Conclusion

In pursuit of providing environmental safeguard to restore food safety and sustaining food security to burgeoning population and combat abiotic pollution, a low cost alternative to exorbitant pollution control strategies remained an absolute priority. The outcome of the present investigation revealed the candidate bacterial isolate Burkholderia sp. as a potent, novel bacterial strain for As mitigation and may be useful, through fulfilment of mass production and field validation protocols, in As decontamination.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work.

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