

## Exploring Arsenic Transformation and Chemotaxis in a *Pseudomonas* sp.

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### Abstract

An arsenic-resistant bacteria (strain PKA 200) was isolated from the Hindon River (Ghaziabad) after analyzing its water sample. Strain PKA 200 belongs to the *Pseudomonas* genus and can convert harmful arsenite [As(III)] into less harmful arsenate [As(V)]. Strain PKA 200 exhibits a remarkable ability to sense and move towards arsenite. Strain PKA 200 is a promising candidate for studying how bacteria sense and transform arsenic. Here, we report a novel circular plate assay to demonstrate both chemotaxis and biotransformation of arsenite by *Pseudomonas* sp. PKA 200.

**Keywords:** Detoxification, Arsenic, Biotransformation, Chemotaxis

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## INTRODUCTION

Arsenic is a naturally occurring metalloid that can be found in the environment, both as a result of natural processes and human activities. It exists in various chemical forms, including organic and inorganic species, which have different levels of toxicity.<sup>1,2</sup> Most common forms are arsenite [As(III)] and arsenate [As(V)]. Arsenite is generally more toxic and mobile than arsenate.<sup>3-5</sup> Biotransformation and chemotaxis play significant roles in the environmental behavior and impact of arsenic.

The arsenic biotransformation involves the microbial conversion of arsenic compounds from one form to another.<sup>6,7</sup> This process can significantly influence the toxicity, mobility, and bioavailability of arsenic in the environment. Microorganisms, particularly bacteria, play a crucial role in arsenic biotransformation.<sup>6,7</sup> They can metabolize arsenic through several pathways, including reduction, oxidation and methylation.<sup>6,7</sup>

Microbial reduction of arsenate [As(V)] to arsenite [As(III)] is a common biotransformation pathway.<sup>8-11</sup> Arsenate-reducing bacteria, such as members of the genus *Shewanella* and *Geobacter*, utilize arsenate as an electron acceptor in anaerobic respiration.<sup>8-11</sup> This reduction increases the solubility and mobility of arsenic, making it more bioavailable and toxic.

Some bacteria can oxidize arsenite to arsenate depending on the specific bacterial species.<sup>12</sup> The oxidation of highly toxic arsenite to less toxic arsenate is considered as a detoxification mechanism.<sup>13,14</sup> These bacteria are crucial in controlling the speciation and mobility of arsenic in the environment. By transforming arsenic compounds, they can influence the bioavailability and toxicity of arsenic to other organisms. Arsenic-oxidizing bacteria are found in a range of environments, including arsenic-contaminated soils, groundwater, surface waters, and hydrothermal vent systems. They are also found in extreme environments like arsenic-rich hot springs.<sup>13,14</sup> This process can immobilize arsenic by converting it to less soluble and less toxic forms. Arsenite-oxidizing bacteria have potential applications in bioremediation efforts to reduce arsenic contamination in water and soil.<sup>13,14</sup> By converting toxic arsenite into less

toxic arsenate, they can help mitigate the health and environmental risks associated with arsenic contamination.<sup>15</sup>

Arsenic can also undergo methylation, a process where methyl groups are added to arsenic compounds.<sup>16</sup> Certain microorganisms, such as *Methanobacterium* and *Methanosarcina*, can methylate arsenic to produce organic forms like monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA).<sup>16-18</sup> Methylation can reduce arsenic toxicity, but it can also produce volatile arsenic compounds, which may lead to environmental dispersion.<sup>16-18</sup>

Some arsenic-oxidizing bacteria exhibit chemotactic responses to different concentrations of arsenic compounds in their environment.<sup>14,15</sup> For example, they might move toward areas with higher concentrations of arsenite to facilitate its oxidation and subsequent detoxification.<sup>15</sup> Alternatively, if arsenate is more favorable for their metabolism, they may exhibit chemotaxis towards regions with higher concentrations of arsenate.<sup>15</sup>

Understanding the interplay between arsenic oxidation and chemotaxis in bacteria is crucial for environmental scientists and microbiologists studying bioremediation strategies or natural attenuation of arsenic-contaminated sites. This communication details the discovery of a new bacterium exhibiting both the ability to convert toxic arsenite into a less harmful form and a remarkable movement towards it. Furthermore, in this communication, we report a novel circular plate assay to demonstrate both chemotaxis and biotransformation of arsenite by a newly isolated *Pseudomonas* sp. PKA 200.

## MATERIALS AND METHODS

### Isolation of Arsenite-resistance bacteria

A water sample from the Hindon River was collected in sterile bottles for isolating arsenite-resistant bacteria. The study used broth culture enrichment followed by selective agar plating with sodium arsenite. Initially, 1 mL sample was added to 500 mL of nutrient broth containing 200 ppm sodium arsenite, and the bacteria were cultured for two days. Following this, the sample underwent serial dilution and was plated on selective agar media supplemented with the same concentration of arsenite. These plates

were then incubated for an additional two days. Ultimately, eight different bacterial isolates, each displaying unique morphologies, were selected, and preserved for further analysis.

### Screening of bacteria for Arsenic resistance

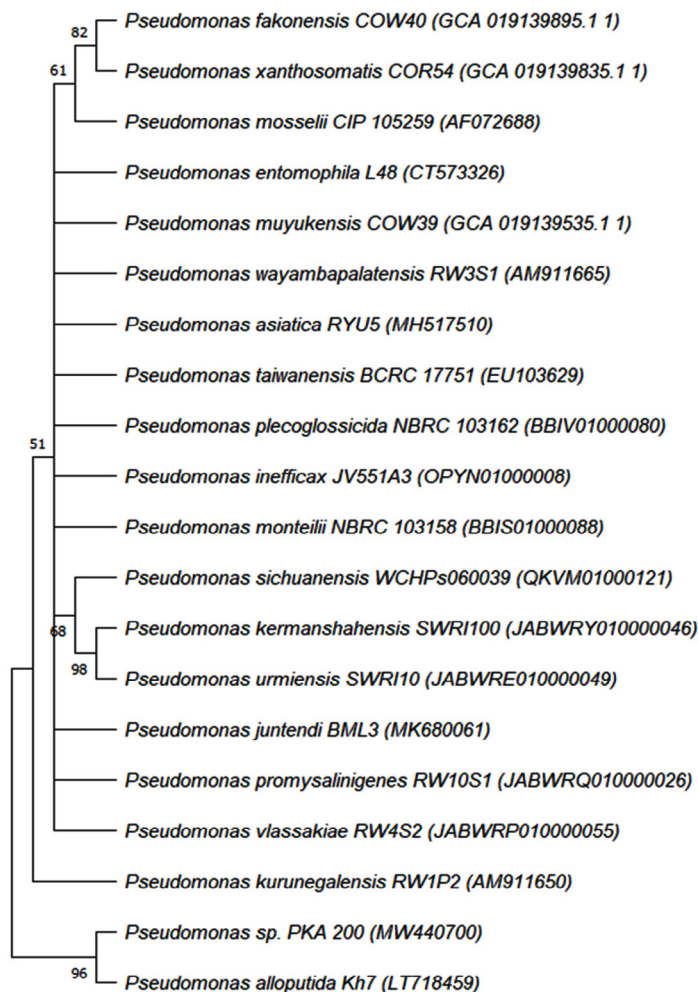
To evaluate the tolerance levels of each bacterial isolate, the eight strains were individually streaked onto nutrient agar plates with sodium arsenite concentrations ranging from 200 to 1000 ppm. Following a 48 hour incubation period at 30°C, only one strain, labeled PKA 200, demonstrated significant resistance, successfully growing even at the maximum concentration of 500 ppm. As a result, strain PKA 200 was selected for further study.

### Identification and biochemical characterization of bacterial strain

The bacterial strain PKA 200 was identified by sequencing its 16S ribosomal RNA gene, utilizing a method described in a previous study.<sup>19</sup> Phylogenetic tree was constructed as described previously.<sup>19</sup> All biochemical tests and morphological characteristics were performed by the standard methods as described previously.<sup>20</sup>

### Arsenite transformation assay

*Pseudomonas* sp. PKA 200 was grown on nutrient agar plates containing 500 ppm sodium arsenite or sodium arsenate. The oxidation of arsenite to arsenate and the reduction of arsenate to arsenite were determined by the silver nitrate method.<sup>21</sup>



**Figure 1.** Phylogenetic analysis of *Pseudomonas* sp. PKA 200

### Determination of minimum inhibitory concentrations

Strain PKA 200 was grown on nutrient agar plates containing different concentrations of arsenite (200 ppm to 1000 ppm) and plates were incubated at 30°C. The MIC was considered as the lowest concentrations of arsenite that inhibits the complete growth of strain PKA 200 within 48 hours.

### Chemotaxis towards Arsenite

Drop plate assay and circular plate assay were used to demonstrate the chemotactic potential of *Pseudomonas* sp. PKA 200 towards arsenite.

### Drop plate assay

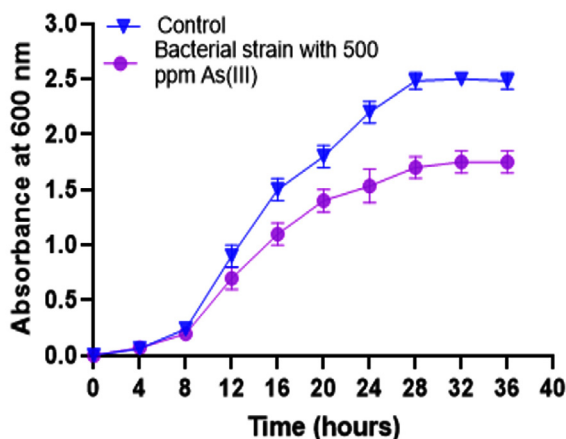
Drop plate assay was performed as described previously.<sup>22</sup> The bacteria were grown overnight in a minimal medium containing 200 ppm sodium arsenite (the attractant) and 20 mM sodium succinate (the energy source). Subsequently, the cells were harvested by centrifugation, washed twice with a saline solution, and resuspended in minimal medium containing 0.3% agar. This cell suspension was then poured onto a Petri plate to solidify. To test if the bacteria are attracted to arsenic, small amounts of sodium arsenite, sodium arsenate, or aspartic acid (a known attractant) were placed in the center of Petri dishes. The dishes were then incubated for a few hours. If the bacteria are attracted, they will gather around the crystals in visible rings.

**Table.** Biochemical and Morphological characteristics of *Pseudomonas* sp. PKA 200

Characteristics	Results
Gram stain	Negative
Shape	Rods
Colony colour	White
Catalase	Positive
Spore formation	Negative
Oxidase	Positive
Motility	Positive
Casein hydrolysis	Negative
Gelatin hydrolysis	Negative
Citrate utilization	Positive
Indole formation	Positive
Urease test	Negative
Growth on MacConkey Agar	Positive
H <sub>2</sub> S production	Negative
Aesculin hydrolysis	Negative
<b>Fermentation of carbohydrates</b>	
Raffinose	Negative
Mannitol	Positive
Arabinose	Negative
Maltose	Negative
Sucrose	Negative
Lactose	Negative

### A circular plate assay

Two rigs of 0.4% bacto agar with increasing concentration of sodium arsenite (100 ppm to 200 ppm) towards center were casted on glass petri-dishes. The outermost ring was inoculated with cells of strain PKA 200, whereas



**Figure 2.** Growth curve of *Pseudomonas* sp. PKA 200 in the absence (control) or presence of sodium arsenite

the inner ring remained uninoculated. This experiment was carried out in triplicate and plates were collected at 0 h, 24 h and 36 h, respectively and flooded with 0.1 M silver nitrate solution. Chemotaxis and transformation of arsenite was determined by the color development with 0.1 M silver nitrate solution.<sup>21</sup> Arsenite provides yellow color with silver nitrate whereas arsenate give red-brown color.<sup>21</sup>

## RESULTS

### Characterization of an Arsenite-oxidizing bacteria

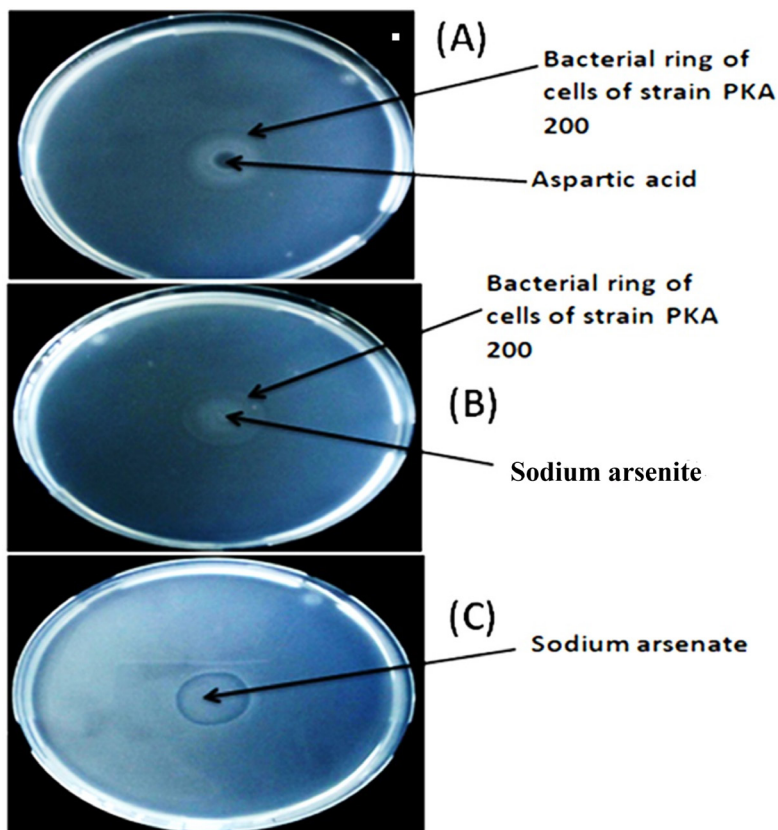
An arsenite-oxidizing bacterium, strain PKA 200 was isolated from a polluted water sample collected from the Hindon river, Ghaziabad. Strain PKA 200 was subjected to the 16S rRNA gene sequencing and identified as a member of genus *Pseudomonas*. The 16S rRNA gene sequence of strain PKA 200 was submitted to the NCBI Genbank under accession number MW440700.

The phylogenetic tree, constructed using the neighbour-joining method, showed that strain PKA 200 forms a clade with *Pseudomonas alloputida*, supported by a high bootstrap value (Figure 1).

Cells of strain PKA 200 were Gram-negative, strictly aerobic, motile and rod shaped. Cells were 1.4-2.0 µm long and 0.7-1.2 µm in diameter. The various biochemical tests of strain PKA 200 were summarized in Table.

### Growth studies

The growth of *Pseudomonas* sp. PKA 200 was assessed under two different conditions: in the presence and absence of arsenite [As(III)]. In this study, bacterial growth was measured by monitoring optical density (OD) at a specific wavelength (600 nm) (Figure 2). In the absence of arsenite, *Pseudomonas* sp. PKA 200 showed better growth, indicating that without the toxic stress of arsenite, the bacteria were able to proliferate efficiently. The normal growth conditions allowed for rapid cell division and biomass accumulation.



**Figure 3.** Chemotaxis of *Pseudomonas* sp. PKA 200. (A) aspartic acid, (B) sodium arsenite, and (C) sodium arsenate

In the presence of arsenite, bacterial growth was significantly reduced. This suggests that arsenite had an inhibitory effect on the metabolism or cellular processes of *Pseudomonas* sp. PKA 200. The bacterium may have been exposed to oxidative stress, disruption in protein synthesis, or impaired energy production, which slowed down or halted its growth. The growth studies highlights how arsenite, as a toxic substance, impacts bacterial growth, and provides insight into the bacterium's potential tolerance limits or mechanisms of resistance. The data in Figure 2 likely show slower growth rates or lower final biomass in arsenite-containing conditions, illustrating the stress imposed by this toxic compound.

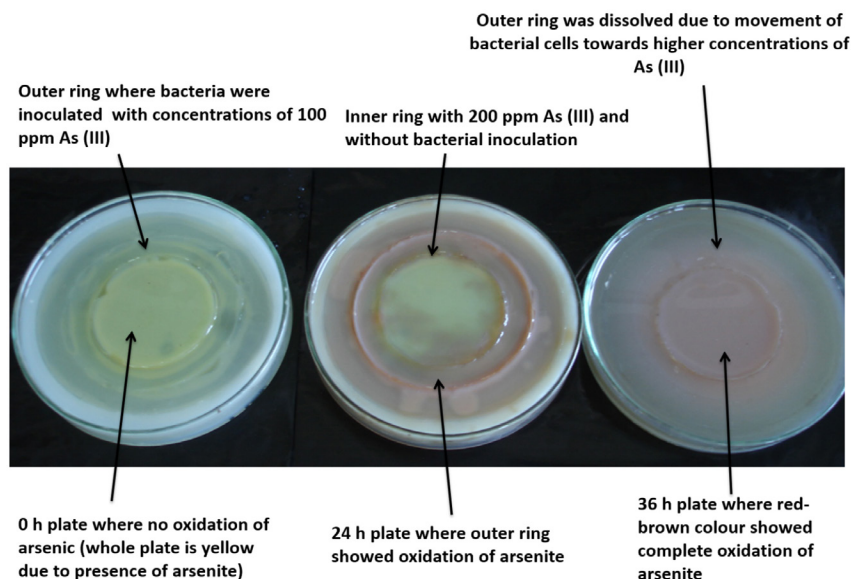
#### Arsenite transformation assay

The potential of *Pseudomonas* sp. PKA 200 for arsenite [As(III)] oxidation and arsenate [As(V)] reduction was determined qualitatively using silver nitrate ( $\text{AgNO}_3$ ) test that is based on reaction between  $\text{AgNO}_3$  and As(III) or As(V). When 0.1M silver nitrate solution was added into 48 h old bacterial nutrient agar culture plate containing 500 ppm sodium arsenite, the plate colour was red-brown due to formation of silver arsenate, confirming complete bacterial oxidation of arsenite [As(III)] to arsenate [As(V)] within

48 h. Whereas, the 48 h old control uninoculated nutrient agar plate containing 500 ppm sodium arsenite produced a yellow colour after adding silver nitrate solution due to presence of silver arsenite. To monitor the ability of cells of strain PKA 200 to reduce arsenate, the silver nitrate solution was added into 48 h old bacterial nutrient agar plate containing sodium arsenate, the plate colour was red-brown due to presence of silver arsenate, suggesting that cells of PKA 200 were unable to reduce arsenate to arsenite. Minimal inhibitory concentration of As(III) for strain PKA 200 was determined as 700 ppm.

#### Chemotaxis towards Arsenite Drop plate assay

Drop plate assay exhibited the bacterial ring of cells of strain PKA 200 around aspartic acid which is known attractant (Figure 3A). Bacterial ring was also observed where chemotactic material was sodium arsenite, suggesting movement of the PKA 200 cells towards arsenite (Figure 3B). However, there is no bacterial ring towards sodium arsenate (Figure 3C). This data indicates that strain PKA exhibited chemotaxis only towards As(III) and did not show chemotactic behaviour towards As(V).



**Figure 4.** A Circular plate assay showing bacterial chemotaxis and transformation towards arsenite

### Circular plate assay

A circular plate assay was carried out to show the capability of strain PKA 200 to oxidize arsenite and chemotaxis towards it at regular intervals. Figure 4 showed that there is no biotransformation and bacterial chemotaxis towards arsenite in 0 h plate (at time of experiment start) and the colour of the plate was yellow after flooding with 0.1 M silver nitrate. In 24 h plate, most of arsenite around the outer bacterial ring was converted to arsenate, indicating by the red-brown colour after flooding with 0.1 M silver nitrate. The red-brown colour was due to formation of silver arsenate (Figure 4). In 36 h plate, bacteria completely moved to higher concentrations of arsenite and transformed into arsenate, confirming by complete red-brown colour of plate (after flooding with 0.1 M silver nitrate) and disappearance of outermost ring where bacteria was inoculated. This assay also suggests that arsenite oxidation was associated with arsenite chemotaxis in *Pseudomonas* sp. PKA 200.

### DISCUSSION

In this study, we have isolated and identified a new arsenite-oxidizing bacterium, *Pseudomonas* sp. PKA 200. Literature studied showed that many As(III) oxidizing bacteria including *Pseudomonas*,<sup>23</sup> *Agrobacterium*,<sup>24</sup> *Alkalilimnicola*,<sup>25</sup> *Microbacterium*,<sup>26</sup> *Micrococcus*,<sup>27</sup> *Bosea*,<sup>28</sup> *Delftia*,<sup>29</sup> *Thiobacillus*,<sup>30</sup> *Anaeromyxobacter*,<sup>30</sup> and *Ensifer*<sup>31</sup> have already been characterized for As(III) oxidation. Few bacteria grew well in arsenic containing media and gained energy during As(III) oxidation.<sup>32-35</sup> As(III) served as a sole electron donor during their growth.<sup>32,35</sup> In this study, the growth of *Pseudomonas* sp. PKA 200 was monitored in presence of As(III) and results showed that *Pseudomonas* sp. PKA 200 was unable to gain energy during As(III) oxidation as there was no inductive effect of As(III) on growth of *Pseudomonas* sp. PKA 200. Our study clearly showed that the growth of strain PKA 200 was reduced in the presence of arsenite as compared to control where no arsenite was present in the media. Furthermore, an arsenic transformation assay using silver nitrate demonstrated that strain

PKA 200 detoxified arsenite [As(III)] into arsenate [As(V)], which is less toxic than arsenite.

In this study, we reported that *Pseudomonas* sp. PKA 200 exhibited chemotaxis toward arsenite. Earlier research indicates that only a few bacteria are known to show positive or negative chemotaxis towards heavy metals.<sup>36,37</sup> Positive chemotaxis of heavy metals is considered as a part of bioremediation mechanism in which bacteria move towards heavy metals to transform them.<sup>36</sup> On the other hand, negative chemotaxis is a survival mechanism in which bacteria remove themselves from heavy metals mediated toxicity areas.<sup>36</sup> Research investigations have reported that four marine strains of *Pseudomonas* species exhibited negative chemotaxis of heavy metals.<sup>37</sup> In this report, the positive chemotaxis towards arsenite by a *Pseudomonas* sp. PKA 200 was demonstrated using various methods including drop plate assay and a circular plate assay. The drop plate assay has already been used for successful demonstration of chemotaxis towards various xenobiotic compounds using *Pseudomonas* species.<sup>38</sup> For example, Arora and Bae<sup>38</sup> reported that *Pseudomonas* sp. strain JHN exhibited positive chemotaxis towards 4-chloro-2-nitrophenol using drop plate assay. Similarly, the five isolates of *Pseudomonas*, taxonomically related to *P. jessenii*, *P. moorei*, *P. vancouverensis*, *P. reinekei*, *P. koreensis*, and *P. moraviensis* exhibited chemotaxis towards sodium dodecyl sulphate.<sup>39</sup> Another strain BUR11 of *Pseudomonas* sp. was reported to exhibit chemotaxis towards parathion, chlorphrifos and their hydrolytic intermediates.<sup>40</sup> Grim and Harwood<sup>41</sup> used drop plate assay to exhibit chemotaxis towards naphthalene by *Pseudomonas putida* G7 and *Pseudomonas* sp. strain NCIB 9816-4.

In this study, we introduce a novel circular plate assay to demonstrate chemotaxis toward arsenite by *Pseudomonas* sp. PKA 200. The assay indicates that arsenite oxidation is associated with chemotaxis in *Pseudomonas* sp. PKA 200. Previous investigations have shown that no single method in the literature can simultaneously assess both chemotaxis and arsenite oxidation.<sup>14,15</sup> Therefore, this assay is the only method that can monitor both arsenite oxidation and chemotaxis toward arsenite. This method can also be used to detect

arsenate reduction and chemotaxis toward arsenate.<sup>42</sup> Additionally, it may be applicable for studying bacterial chemotaxis and the reduction or oxidation of other heavy metals, such as the reduction of selenite to red-colored selenium and chemotaxis toward it.<sup>43</sup> This method could also be suitable for investigating bacterial degradation of colored organic compounds, such as nitrophenols and chloronitrophenols.<sup>44</sup>

To date, only few As(III) oxidation bacteria exhibited chemotaxis towards arsenite.<sup>14,15</sup> The common feature of these bacteria is that they gained energy during arsenite oxidation. However, in this study *Pseudomonas* sp. PKA 200 exhibited chemotaxis towards arsenite, but it was unable to gain energy during arsenite oxidation.

## CONCLUSION

*Pseudomonas* strain PKA-200 shows a distinctive capability to biotransform the toxic arsenite [As(III)] into less harmful arsenate [As(V)]. Its unique ability to sense and move toward arsenite underscores its potential as an effective agent in bioremediation of arsenic-contaminated environments. This strain holds significant promise for further research into microbial arsenic sensing and detoxification mechanisms, offering a valuable foundation for developing sustainable biotechnological solutions for arsenic mitigation.

## ACKNOWLEDGMENTS

None.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

## FUNDING

None.

## DATA AVAILABILITY

All datasets generated or analysed during this study are included in the manuscript.

## ETHICS STATEMENT

Not applicable.

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