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Isolation and Identification of Arsenic Hyper-Tolerant Bacterium with Potential Plant Growth Promoting Properties from Soil

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Abstract: The soil and groundwater of the Bhagobangola I block of Murshidabad district, West Bengal, India is severely arsenic-contaminated. A bacterium was isolated from the garden soil of the Mahishasthali village, which could tolerate 36.49 mM arsenic (III), 280.44 mM arsenic (V) and 63 mM chromium (III), which makes it arsenic (III and V) and chromium (III) hyper-tolerant bacterium. The growth pattern of this bacterium does not show much alteration in the presence of 10 mM arsenic (III) and chromium (III), emphasizing its resistance to these heavy metals. Scanning electron microscopic analysis depicted this bacterium to be rod-shaped with a size of ~1.45 µm. 16S rDNA sequencing, followed by subsequent phylogenetic analysis, established the identity of this bacterium as *Microbacterium paraoxydans*. This bacterium is capable of bioremediation of arsenic and showed 30.8% and 35.2% of bioremediation for 1mM and 22.6%, and 30.5% of bioremediation for 4mM arsenite, over a period of 24 and 48 h, respectively. *Microbacterium paraoxydans* also exhibits potential plant growth-promoting properties such as nitrogen fixation, phosphate solubilization, indole-3-acetic acid production and production of siderophores. Therefore, the heavy metal resistance, bioremediation potential and plant growth-promoting potential of the bacterium could be utilized not only for reduction in arsenic toxicity in soil and groundwater but also for plant growth promotion.

Keywords: arsenic hyper-tolerant bacterium; 16S rDNA sequencing; *Microbacterium paraoxydans*; arsenic bioremediation; plant growth-promoting bacterium

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1. Introduction

At present, arsenic toxicity in soil and groundwater is a global problem. Arsenic exists in nature in both inorganic and organic forms. Arsenic inorganic forms include arsenite (+3), arsenate (+5), arsenic (0) and arsenide (−3). Arsenic organic forms include monomethyl arsonic acid (MMA), dimethyl arsonic acid (DMA), arsenobetaine and arsenocholine. Arsenite has high solubility, mobility and bioavailability, and it is 100 times more toxic than arsenate. Arsenite inhibits the action of many enzymes responsible for biochemical reactions within the human body. Arsenic pollution may have anthropogenic sources in addition to a geogenic origin. The anthropogenic sources of arsenic include pesticides, fungicide, weedicide, wood preservative, etc. Nine districts of West Bengal have arsenic-contaminated soil and groundwater. Murshidabad district is worst affected by arsenic toxicity. Bhagobangola I and II, Lalgola, Beldanga I, Jalangi, Domkal and Hariharpara blocks of the Murshidabad district, on the eastern bank of Bhagirathi river, show

much higher arsenic contamination of soil and groundwater than the blocks on the western bank. The problem of arsenic toxicity in the Murshidabad district is mainly geogenic in origin, as evident from the fact that Ganga-Brahmaputra-Meghna delta is one of the worst arsenic-contaminated regions of the world [1–12].

Due to their residence in highly arsenic-contaminated soil and groundwater, some bacteria develop a tolerance to arsenic. Arsenite oxidase operon aox*ABCD* and Arsenate reductase operon ars*RBC*, ars*RABC* and ars*RDABC* of these bacteria, have genes clustered for arsenic hyper-tolerance. These operons code for different metal regulatory proteins, metallic chaperones, ATPases, arsenic exporters, importers, reductases, oxidases, methyltransferases, etc. The aforementioned proteins take part in various biochemical reactions and metabolic processes, which include the transportation and sequestration of arsenic in different compartments of the cell, detoxification or transformation of arsenical compounds by oxidation, reduction, methylation or demethylation, etc. The bacterial membrane plays a significant role in arsenic hyper-tolerance. Some proteins participate in arsenicrelated biochemical processes and adsorb it on the bacterial membrane, thereby inhibiting its entry inside the bacterial cell [13]. Chromium (III) is essential for the metabolism of glucose and lipids. However, a large concentration of chromium (III) in the body may cause health hazards including lung cancer [14]. Bioremediation is a well-established technology used for the mitigation of heavy metal toxicity and xenobiotic stress of the environment by application of various biological agents. The bioremediation process could be mediated by bacteria, plants (phytoremediation), fungi (mycoremediation), etc. Biotransformation (transformation of the pollutant into a less toxic form), biodegradation (degradation of the pollutant into simpler harmless compounds), bioaccumulation/bioadsorption (accumulation or adsorption of the pollutant by plants or bacteria), biovolatilization (release of the pollutant into the atmosphere in gaseous form) are some aspects of bioremediation [15–18]. The process of bioremediation is dependent upon biological agents, indigenous or exotic to the local environment. Multiple abiotic factors (temperature, pH, nutrient, oxygen content, etc.) of the site, concentration, type and bioavailability of the pollutant are important contributors to the heterogeneous environment, with respect to bioremediation, thereby affecting the microbe-mediated bioremediation process, either directly or indirectly. Extrinsic factors, such as modern anthropogenic activities, could also transform the microbiogeochemical environment of the site. In addition to this, biotic factors, such as microbial communities present in the local environment, their density, composition and interplay, might significantly alter or impact the process of bioremediation [18–22].

The application of bacteria as a biofertilizer has turned out to be an effective as well as an eco-friendly way of reducing the use of chemical fertilizers. The promotion of plant growth is usually accomplished by the production of siderophores, indole-3-acetic acid (IAA), solubilization of phosphate, zinc, potassium, biological nitrogen fixation and production of ACC deaminase by the bacteria. Biofertilizers could play an important role in maintaining sustainable agriculture and industrial biotechnology [23]. Microorganisms like *Azotobacter* participate significantly in the fixation of atmospheric nitrogen (by an aerobic process), production of plant hormones, solubilization of insoluble phosphate and reduction in harmful effects of phytopathogens and xenobiotics, thereby resulting in better production of crops such as wheat, barley, rice, oat, etc. [24]. The plant growth-promoting bacteria recognize their suitable host and colonize the plant roots to increase the growth of plants either directly (by nitrogen fixation, plant hormone production, insoluble phosphate solubilization, etc.) or indirectly (by enhancing the tolerance limits of plants to various toxic substances). Despite the fact that the application of bacterial biofertilizers is one of the best ways of enhancing plant growth, extensive research is needed, mainly in agricultural fields in the presence of multiple variable abiotic and biotic factors, to decipher the best way of using bacterial biofertilizer [25].

In West Bengal, the extent of arsenic toxicity in groundwater and agricultural fields, physicochemical parameters participating in arsenic mobilization and effects of long-term exposure to arsenic (by consumption of arsenic-contaminated drinking water and cereals,

and working in arsenic-contaminated fields) on human health, were investigated by many research groups [26–29]. However, the presence of different microbial communities and their potential role in the reclamation of arsenic-contaminated groundwater and the soil needs further investigation, particularly in the Murshidabad district. The potential role of indigenous heavy metal hyper-tolerant bacteria, in plant growth promotion in arsenic-contaminated agricultural fields, is yet to be deciphered completely. The current study aims to isolate heavy metal hyper-tolerant bacterium from arsenic-contaminated garden soil of the Bhagobangola I block of the Murshidabad district. The heavy metal hyper-tolerance and plant growth-promoting potential of the isolate have been established in this study. The role of the heavy metal hyper-tolerant bacterium in the bioremediation of arsenic has been predicted. The arsenic bioremediation potential of the bacterium could be used for designing a biofilter, in order to reduce arsenic concentration in wastewater and groundwater. In addition to this, the application of the isolated bacterium as a potential biofertilizer might increase the crop yield.

2. Materials and Methods

2.1. Selection of the Study Area

Groundwater and soil samples of the Bhagobangola I block of the Murshidabad district are highly arsenic-contaminated. The arsenic contamination of aquifers in this block is mostly geogenic in origin [11,12]. Garden soil samples were collected from the Mahishasthali village (Latitude—24.334230 and Longitude—88.310950) of Bhagobangola I block, for biochemical characterization and isolation of arsenic hyper-tolerant bacteria.

2.2. Physicochemical Analysis of the Soil Sample

A total of 100 mg of the garden soil collected from the Bhagobangola I block was dissolved in 100 mL of double-distilled water to achieve a concentration of 1 mg/mL. A Soil and Water Testing Kit (ORLAB, Hyderabad, India) was used for the estimation of pH, iron and fluoride. Arsenic and phosphate were estimated using Arsenic Testing Kit (Merck, Darmstadt, Germany) and spectrophotometrically using ammonium molybdate, respectively. The absorbance of the molybdenum blue complex was measured at 690 nm and the concentration of phosphate in the sample was determined by a standard curve of known phosphate concentration.

2.3. Isolation and Characterization of Arsenic Hyper-Tolerant Bacteria

The garden soil sample collected from the Mahishasthali village was contaminated with ~0.1 mg/L (100 ppb) arsenic. For isolation of arsenic hyper-tolerant bacteria, the soil sample (1 mg/mL) was serially diluted and plated on LB agar medium, in presence of increasing concentrations of sodium arsenite up to 500 ppm (3.85 mM). One arsenic hyper-tolerant bacterium (DMAB*) was selected for the downstream work. This bacterium could form colonies in 7.70 mM (1000 ppm) sodium arsenite. The ability to ferment various carbohydrates, such as glucose, sucrose, fructose, lactose, mannitol, inositol and mannose, was also checked by following the standard protocol [30]. The sensitivity of the isolate towards commonly used antibiotics was tested. They were cultured in LB media supplemented individually with the recommended dose of ampicillin (100 µg/mL), chloramphenicol (25 µg/mL), streptomycin (50 µg/mL), rifampicin (100 µg/mL), gentamycin (16 µg/mL), ciprofloxacin (5 µg/mL), vancomycin (30 µg/mL), ofloxacin (2 µg/mL), tetracyclin (10 µg/mL), kanamycin (50 µg/mL) and bleomycin (40 µg/mL) for 24 h at 37 °C. The bacterial growth was tested after the incubation period by turbidometric method [31].

2.4. Scanning Electron Microscope Imaging

The bacterium cultured in LB was fixed in 3% glutaraldehyde (in 0.1 M phosphate buffer, pH 7.2) for 1 h. Three washes were given to the fixed cells and they were postfixed in 1% osmium tetraoxide for 1 h. The cells were dehydrated in increasing concentrations of alcohol 30%, 50%, 75%, 90% and 100% for 5 min. Bacterial cells were filtered

using 0.2 µ black polycarbonate filter. The cells were filter mounted onto SEM stub and sputter coated with 10 nm gold. The coated cells were observed under 15 kV scanning electron microscope.

2.5. 16S rDNA Sequencing and Phylogenetic Analysis for Identification of the Bacterial Isolate

Genomic DNA was isolated from the pure culture pellet of DMAB*. Its quality was evaluated on agarose gel by observation of a single band of high-molecular-weight genomic DNA. The fragment of the 16S rDNA gene was amplified by 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (TACGGTTACCTTGTTACGACTT) primers. On agarose gel, one discrete 1500 bp PCR amplicon band was observed. The contaminants were removed from the PCR amplicon. Forward and reverse DNA sequencing reaction of the PCR amplicon was carried out with 27F and 1492R primers, using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer (Thermo Fisher Scientific Corporation, Waltham, MA, United States). The consensus sequence of the 16S rDNA gene was generated from forward and reverse sequence data using aligner software. The 16S rDNA gene sequence was used to carry out BLAST using the 'nr' database of NCBI GenBank database. Based on the maximum identity score, highest query coverage and lowest E-value, 15 sequences were selected and aligned with rDNA sequence of DMAB*, using the multiple sequence alignment software program Clustal W of MEGA X. The evolutionary history and evolutionary distances were determined by the Neighbor-Joining method and Jukes-Cantor method, respectively. The phylogenetic tree was constructed using MEGA X [32–34].

2.6. Determination of Heavy Metal Resistance

The bacterial isolate was tested for its resistance to heavy metals such as Cu^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Hg^{2+}, Fe^{2+}, Zn^{2+}, Mn^{2+} and Pb^{2+}. A total of 500 mM stock solutions of $CuSO_{4.5}H_2O$, $CoCl_{2.6}H_2O$, $MnSO_4H_2O$, $FeSO_{4.7}H_2O$, $ZnSO_{4.7}H_2O$ and 100 mM stock solution of $CdCl_2H_2O$, $PbCl_2$, $NiCl_{2.6}H_2O$ and $HgCl_2$ were prepared. The heavy metal salts were diluted to obtain the desired concentration. The resistance and susceptibility of the bacterium were determined by inoculating a single isolated colony of the bacterium (from fresh LB-Agar plates) in LB media and by streaking on LB-Agar media, both amended with variable concentrations of the heavy metals starting from 0.5 mM. The bacterial growth was detected after incubation for 24 h at 37 °C. The bacterial growth was tested in LB medium by turbidometric method and in LB-Agar medium by the formation of a bacterial colony [35,36].

2.7. Determination of Minimum Inhibitory Concentration of Arsenic (III and V) and Chromium (III and VI)

For the determination of Minimum Inhibitory Concentration (MIC), a single isolated colony of the bacterium was inoculated in Luria Broth (LB) media supplemented individually, with increasing concentrations of sodium arsenite, sodium arsenate, potassium chromate and potassium dichromate starting from 3.84 mM, 1.60 mM, 5 mM and 0.5 mM, respectively. The bacterial growth was determined in the LB medium by the turbidometric method.

2.8. Study of the Growth Curve

The bacterium was inoculated into LB media, individually containing 10 mM arsenite and 10 mM chromium (III). The LB medium devoid of heavy metal served as the media for control culture. The growth patterns of these three aforementioned cultures were monitored by measuring the optical density at 600 nm. For obtaining the bacterial growth curve, experiments were performed in triplicates. The graph was constructed in Excel with error bars showing the standard deviation.

2.9. Bioremediation Test

The bioremediation test was carried out by inoculation of the arsenic hyper-tolerant bacterium in LB media containing two different concentrations of sodium arsenite, 1 mM and 4 mM. The control culture was inoculated in the LB medium without any arsenic stress. All the sets were incubated under stirring conditions (160 rpm) at 37 °C for 24 h. After incubation, the bacterial cells were separated from the media by centrifugation at 10,000 rpm for 5 min. The arsenic concentration of the media was estimated by SDDC (Silver Diethyldithiocarbamate) method after bioremediation [37].

2.10. Nitrogen Fixation and Phosphate/Potassium Solubilzation by the Bacterial Isolate for Plant Growth Promotion

The ability of DMAB* to fix atmospheric nitrogen in the soil was tested by culturing the bacterium on Jensen's agar medium (a nitrogen-deficient medium) at 37 °C for 48 h. The potential of the bacterium to solubilize insoluble phosphorus and potassium was tested on Pikovskaya agar (with an insoluble source of phosphate) and Aleksandrow agar (with an insoluble source of potassium) media, respectively, at 37 °C for 48 h. The bacterial growth was assessed by the formation of bacterial colonies on the respective agar plates. The formation of a halo around the bacterial colonies on Pikovskaya or Aleksandrow agar media indicates the ability of the bacterium to solubilize insoluble sources of phosphate or potassium, respectively. Bromothymol blue was used as a pH indicator for Jensen's agar medium. The change in color of the medium, from green to blue, indicates towards the increase in pH due to the formation of ammonia, as a result of atmospheric nitrogen fixation by the bacterium [38–41].

2.11. Indole-3-Acetic Acid Production

Pure and fresh bacterial inoculum was added to the LB medium containing 0.5 mg/L of tryptophan. The culture was incubated at 28 °C with continuous shaking at 125 rpm for 48 h. Next, 2 mL of culture was centrifuged at 15,000 rpm for 1 min. Then, 1 mL supernatant was mixed with 2ml Salkowski's solution. The mixture was incubated at room temperature in dark conditions. The absorbance of the developed pink color was measured spectrophotometrically at 530 nm. The concentration of IAA produced by the bacterium was determined from the standard curve of variable concentration of pure IAA [42].

2.12. Siderophore Production

10 µL of fresh culture (10^8 CFU/mL) was spotted on the chrome azurol sulphonate (CAS) agar plate. The plate was incubated at 28 °C for 72 h. The change in color of the CAS agar medium, from blue to orange, indicated siderophore production. For the quantitative assay, 0.5 mL of bacterial LB culture supernatant was mixed with 0.5 mL CAS reagent and incubated for 5 min. The absorbance at 630 nm was measured against a reference of uninoculated broth. The percent siderophore unit (psu) in the culture supernatant was calculated using the formula given below:

$$\text{Siderophore production (psu)} = [(A_r - A_s)/A_r] \times 100$$

where A_r = absorbance of the reference and A_s = absorbance of the sample [43].

3. Results and Discussion

The Mahishasthali village of Bhagobangola I block has soil and groundwater samples contaminated with high concentrations of arsenic, often beyond the maximum permissible limit of arsenic in drinking water given by WHO. WHO recommends 10 µg/L (10 ppb) as the maximum permissible limit of arsenic in drinking water. However, ~60% of groundwater samples are contaminated with greater than 10 µg/L (10 ppb) of arsenic in Bhagobangola I block. A garden soil sample contaminated with arsenic (~0.1 mg/L (100 ppb)) was collected from Mahishasthali village and used for downstream work. In our previous study, we have reported about two arsenic-resistant bacteria isolated

from the agricultural soil of Asanpara village [11,12,44]. In this study, an arsenic hyper-tolerant bacterium was isolated from the garden soil of the Mahishasthali village and reported to show heavy metal tolerance and plant growth-promoting properties. To the best of our knowledge, this is the first study from the Murshidabad district to report the isolation and characterization of heavy metal hyper-tolerant bacterium with plant growth-promoting potential.

3.1. Biochemical Characterization of the Garden Soil

Garden soil was collected from the part of Mahishasthali village near the Bhagobangola I railway station (Figure 1). The pH of the soil is in a slightly basic range. The phosphate, although present in low amounts (0.02 mg/L), might allow for higher solubility, mobility and leachability of arsenic. The presence of iron (0.03 mg/L) in the soil allows for the existence of arsenic in the form of ferrous arsenate, ferric arsenate and ferric arsenite. Most importantly, the presence of arsenic in ~0.1 mg/L (100 ppb) concentration in the garden soil is a matter of concern, as arsenic might get incorporated into crops/vegetables grown in the soil and gets biomagnified in the food chain. However, the presence of another toxic element, fluoride, could not be detected in the soil (Table 1) [45–47].

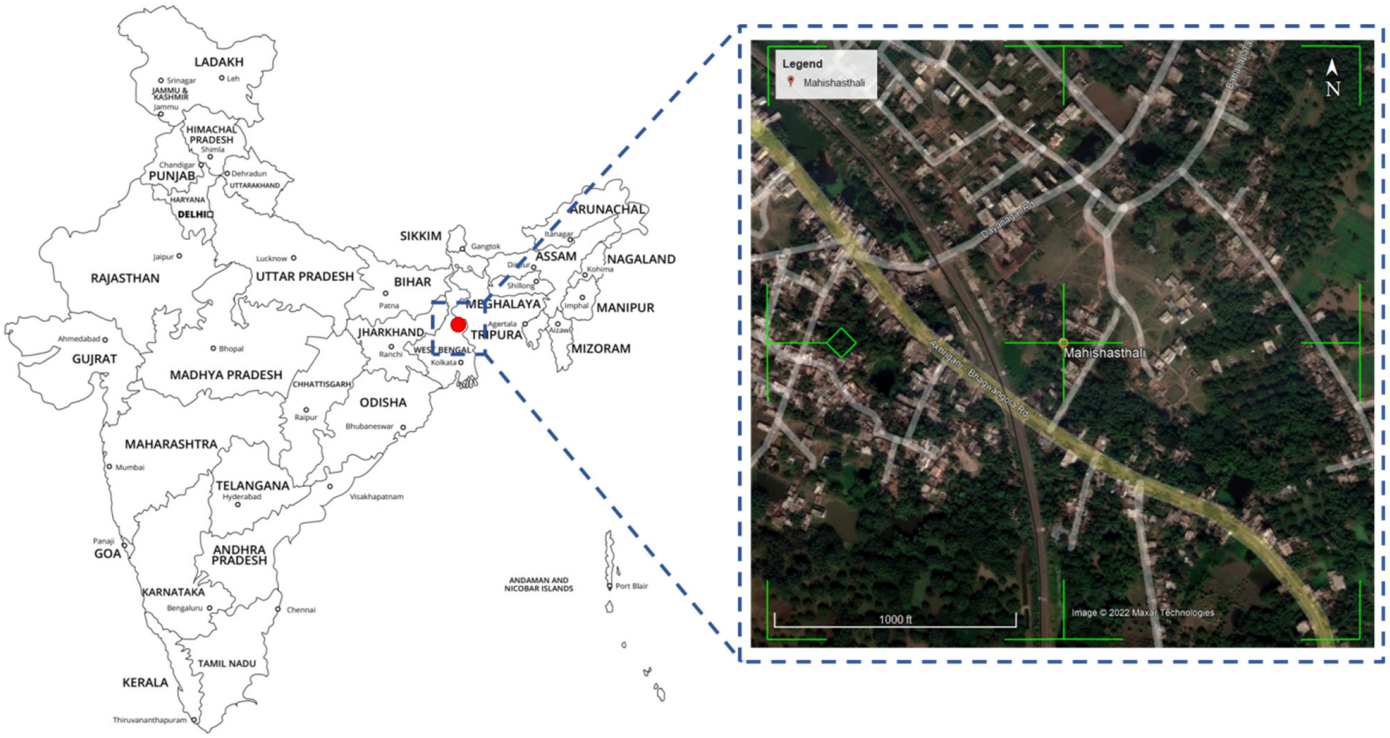

Figure 1. Map of the study area in Mahishasthali village of Bhagobangola I block of Murshidabad district. The map of India was taken freely from the website https://worldmapwithcountries.net/2020/03/12/india-map-with-states/ (accessed on 11 July 2022). The map location of the study area was made in Google Earth Pro.

Table 1. Physicochemical parameters of the soil sample.

Physicochemical Parameters	Concentration (mg/L)
pH	7.5
Iron	0.03
Phosphate	0.02
Arsenic	~0.1
Fluoride	0

3.2. Isolation and Characterization of Arsenic Hyper-Tolerant Bacterium from the Garden Soil

The arsenic hyper-tolerant bacterium was isolated from the garden soil by serial dilution of the soil sample and subsequent plating in LB agar plates, amended with increasing concentration of sodium arsenite. On LB agar plates, the isolated bacterium formed round, yellow-pigmented mucoid colonies of moderate size (about 1 mm) (Figure 2A, Table 2) [48]. The bacterium DMAB* is Gram-positive and acid-fast-negative in nature. Scanning electron microscopy confirmed that the bacterium is having a rod shape with a length of ~1.45 µm (Figure 2B, Table 2). The aforementioned findings corroborate the characteristics of different species of *Microbacterium* characterized by Qian et al., Jung et al. and Hadjadj et al. [49–51]. The isolated bacterium was sensitive to the recommended dose of common antibiotics such as ampicillin, chloramphenicol, tetracycline, gentamycin, streptomycin, ofloxacin, ciprofloxacin, rifampicin, vancomycin and kanamycin. However, the bacterium is resistant to the recommended dose of anti-tumour antibiotic bleomycin (Table 3). This bacterium could ferment common sugars such as glucose, fructose, sucrose and mannitol but not lactose, mannose and inositol (Table 4). Since this bacterium showed tolerance to a very high concentration of arsenite and arsenate (much higher than the average MIC (~20 mM arsenite and 100 mM arsenate) usually observed for the genus *Microbacterium*), it was considered to be hyper-tolerant to arsenic [52].

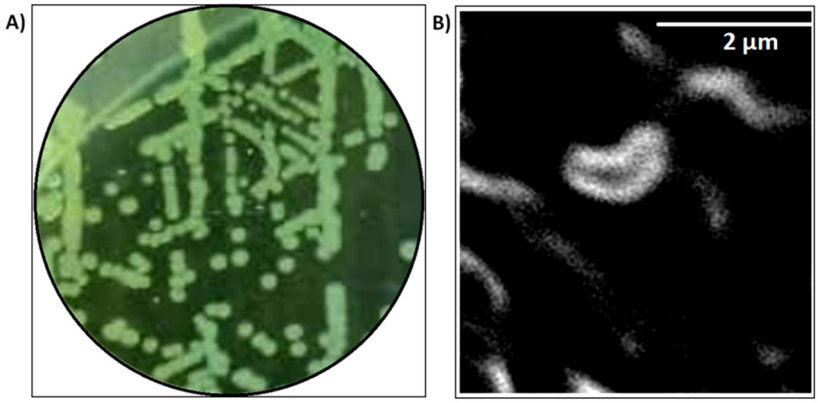

Figure 2. (**A**) Isolation of arsenic hyper-tolerant bacterium from arsenic-contaminated garden soil. Colonies of DMAB* cultured in LB-agar medium supplemented with 7.70 mM (1000 ppm) sodium arsenite. (**B**) Scanning electron microscope (SEM) image of DMAB* (*Microbacterium paraoxydans*). SEM analysis showed DMAB* is a rod-shaped bacterium with length ~1.45 µm.

Table 2. Morphological profile of the isolated bacterial strain.

Colony Morphological Profile	
Form	Round
Color	Yellow-pigmented
Texture	Mucoid
Size	Moderate (about 1 mm)
Microscopic Morphological Profile	
Gram's nature	Gram-positive
Acid-fast	Negative
Shape	Rod
Size	1.45 µM

Table 3. Antimicrobial susceptibility/resistance profile of the isolated bacterial strain.

Antimicrobial Agent	Concentration Used (µg/mL)	Susceptibility or Resistance
Ampicillin	100	Susceptible
Bleomycin	40	Resistant
Chloramphenicol	25	Susceptible
Ciprofloxacin	5	Susceptible
Gentamycin	16	Susceptible
Kanamycin	50	Susceptible
Ofloxacin	2	Susceptible
Rifampicin	100	Susceptible
Streptomycin	50	Susceptible
Tetracycline	10	Susceptible
Vancomycin	30	Susceptible

Table 4. Fermentation of different carbohydrates by the bacterial isolate.

Carbohydrates	Fermentation Ability
Glucose	+
Fructose	+
Mannose	–
Sucrose	+
Lactose	–
Mannitol	+
Inositol	–

3.3. 16S rDNA Sequencing and Phylogenetic Analysis Identified the Bacterium as Microbacterium Paraoxydans

The 16S rDNA sequencing followed by phylogenetic analysis identified bacterium DMAB* as *Microbacterium paraoxydans.* Different strains of *Microbacterium paraoxydans* such as the strains DSM 15019, DSM 1920, BLY and MA-25, were detected as close neighbors of DMAB*. The partial sequence of 16S rRNA gene of *Microbacterium paraoxydans* strain DSM 1920, showed the highest identity (99.60%) and query coverage (100%) with the rDNA sequence of DMAB*. In the genome assembly, chromosome I of *Microbacterium paraoxydans* strain DSM 15019, showed 99.46% identity and 100 % query coverage with the rDNA sequence of DMAB* (Figure 3). Different members of the genus *Microbacterium* were reported as arsenic-tolerant bacteria throughout the world. Highly arsenic-resistant *Microbacterium paraoxydans* strains were isolated from textile effluent wastewater of Jaipur, Rajasthan, India. The four isolates (*Microbacterium paraoxydans* strain 3109, *Microbacterium paraoxydans* strain CF36, *Microbacterium* sp. CQ0110Y and *Microbacterium* sp. GE1017) of the study showed MIC of 8–9 g/L of sodium arsenite [53]. These strains also showed resistance to zinc, chromium, selenium and other heavy metals and a sensitivity towards mercury, cadmium, etc [53]. Arsenic-resistant *Microbacterium* sp. strain SZ1 was isolated from arsenic-bearing gold ores of Malaysia [54]. *Microbacterium spp*. isolated from Creven Dol Mine, Allchar, North Macedonia- survived in presence of 209 mM arsenite and 564 mM arsenate [55]. Multiple chromosomally or plasmid-encoded genes are responsible for the arsenic hyper-tolerance potential of bacteria, such as *E.coli*, *Pseudomonas aeruginosa* PAO1 strain, *Acidiphilium multivorans* AIU 301, *Serratia marcescens*, *Acidithiobacillus ferrooxidans*, etc [56]. Predominant genes responsible for arsenic hyper-tolerance are *arsA*, *arsB*, *arsC*, *arsD*, *arsR*, *arsM* and *arsH*, which together form an operon activated by the presence of

arsenic. These genes encode for multiple proteins such as ATPase (*arsA*), membrane-bound efflux pump (*arsB*), arsenate reductase (*arsC*), mettalochaperone (*arsD*), transcriptional repressor (*arsR*), S-adenosylmethionine methyltransferase (*arsM*) and oxidoreductase (*arsH*). ATPase and membrane-bound efflux pump together participate in the translocation of the oxyanion out of the cell, whereas cytoplasmic arsenate reductase converts arsenate to arsenite. The metallochaperone transfers arsenite to the ATPase and enhance its extrusion. The methyltransferase is responsible for methylation of arsenic species and contributes towards its detoxification [52]. In addition to these, other genes such as *aoxA, aoxB, aoxR* and *aoxS*- are also part of arsenic hyper-tolerance and defense mechanism, particularly in *Herminiimonas arsenicoxydans, Agrobacterium tumefaciens, Pseudomonas stutzeri* strain *GIST-BDan2* (EF429003), etc. *aoxA* and *aoxB* genes, together, encode for arsenite oxidase, an enzyme involved in the conversion of arsenite into a relatively less toxic and less mobile form, arsenate. *aoxS* and *aoxR,* together, form a two-component signal transduction system and encode for a sensor kinase and a positive regulator of *aoxAB* operon, respectively [57,58]. Arsenate reductase and arsenic dependent transcriptional repressor is reported in *Microbacterium* sp. strain A33 [59]. ArsR-ArsC fusion proteins are also reported in the genus *Microbacterium* [56]. The presence of *arsB* gene is reported in *Microbacterium paraoxydans* DSM 15019 [52]. Arsenate reductase, arsenical responsive transcriptional repressor, arsenic efflux pump, thioredoxin reductase and an arsenicalresistant protein is present in *Microbacterium* sp. strain SZ1 from gold ores contaminated with arsenic [54]. However, further investigation is required to decipher the arsenic defense mechanisms in detail, in the genus *Microbacterium*.

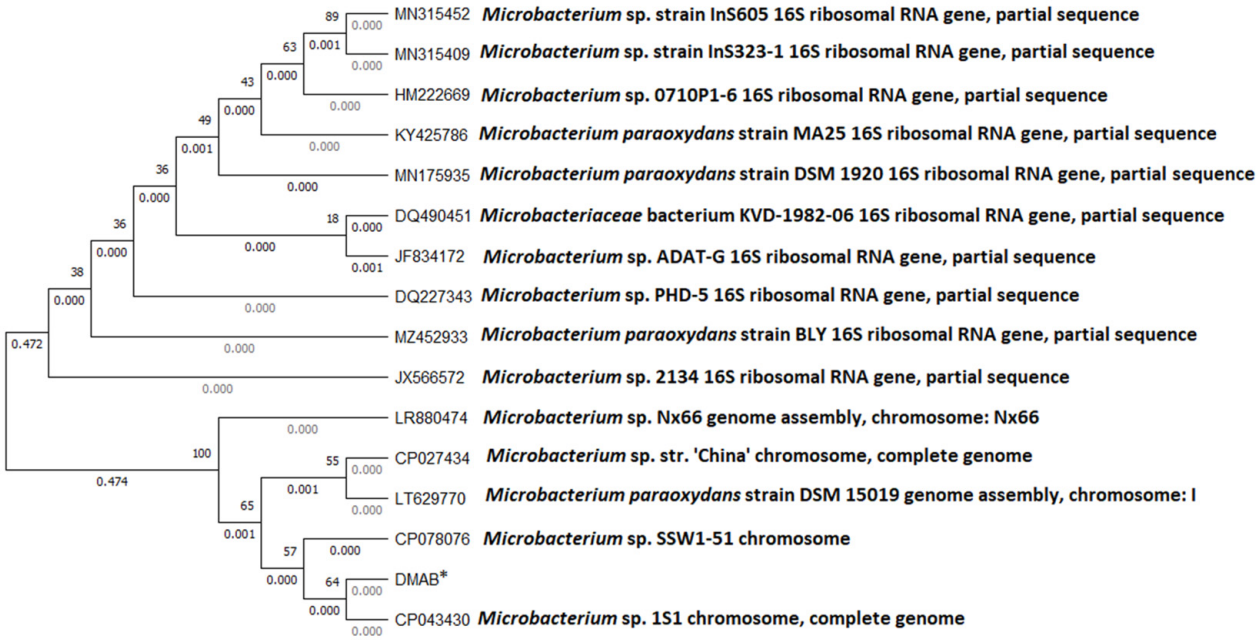

Figure 3. Phylogenetic tree of the bacterial isolate DMAB* (*Microbacterium paraoxydans*). The evolutionary history and evolutionary distances were determined using the Neighbour Joining method and Jukes-Cantor method, respectively. The phylogenetic tree was constructed using MEGA X and includes15 closest homologues of DMAB* identified by BLAST. The branch length and the bootstrap values are shown next to the branches in the figure.

3.4. Heavy Metal Tolerance and Susceptibility of the Isolated Bacterium

The isolated *Microbacterium paraoxydans* could tolerate moderately high concentrations (5 mM) of some heavy metals such as zinc (Zn^{2+}), copper (Cu^{2+}) and manganese (Mn^{2+}). It could also survive in high concentrations of iron (Fe^{2+}) (10 mM). However, the isolate was susceptible towards low concentrations of cobalt (Co^{2+}) and lead (Pb^{2+}) (3 mM and 2 mM, respectively), and even more sensitive towards cadmium (Cd^{2+}), Nickel (Ni^{2+}) and mercury

(Hg^{2+}) (1 mM, 1mM and 0.5 mM, respectively) (Table 5). This strain of *Microbacterium paraoxydans* also showed very high MIC for arsenite (36.95 mM), arsenate (280.44 mM) and chromium (Cr^{3+}) (63 mM). In contrast to this, the MIC for chromium (Cr^{6+}) (the most toxic form of chromium) was only 1 mM for this bacterium (Table 6). The potential of this bacterial isolate to tolerate a very high concentration of arsenite and arsenate was higher than the average MIC (~20 mM arsenite and 100 mM arsenate) usually observed for the genus *Microbacterium* [52]. The arsenic tolerance shown by the isolated strain of *Microbacterium paraoxydans* could be well corroborated with some arsenic hyper-tolerant bacteria isolated from the soils of West Bengal. For example, *Planococcus* KRPC10YT isolated by Chowdhury et al. could survive in 30 mM arsenate and 20 mM arsenite. Both *Bacillus* sp. and *Aneurinibacillus aneurinilyticus* isolated by Dey et al. could tolerate arsenate concentrations up to 4500 ppm and arsenite concentrations up to 550 ppm [60,61]. *Microbacterium* sp. strain 1S1, isolated from the industrial wastewater of Sheikhupura, Pakistan, could tolerate up to 75 mM arsenite and 520 mM arsenate. This strain of *Microbacterium* sp. showed MIC of 3 mM/mL, 4 mM/mL, 4 mM/mL, 5 mM/mL, 5.5 mM/mL, 6 mM/mL and 8 mM/mL, for multiple heavy metals such as selenium, cadmium, nickel, chromium, lead, mercury and cobalt, respectively [62]. In another study, *Microbacterium* sp. AE038-20 showed tolerance to 40 mM arsenite, 100 mM arsenate, 2 mM chromium (Cr^{6+}), 4 mM copper (Cu^{2+}) and 1 mM cadmium (Cd^{2+}) [63]. Therefore, many species of the genus *Microbacterium* could tolerate arsenite and arsenate at a very high concentration. Lead-tolerant *Microbacterium paraoxydans* BN-2 was isolated from the rhizosphere of *E. camaldulensis*, from lead-contaminated soils of BoNgam mine, Thailand [64]. *Microbacterium paraoxydans* strain VSVM IIT (BHU) isolated and discovered by Singh and Mishra showed tolerance towards 200 mg/L of chromium (Cr^{6+}) and 99.96% removal efficiency in presence of 50 mg/L of chromium (Cr^{6+}) [65]. Therefore, the genus *Microbacterium* consists of numerous heavy metal-resistant species, which were reported to be resistant to multiple heavy metals such as arsenic, chromium, lead, copper, cadmium, etc., and different species of the genus *Microbacterium* in general, and *Microbacterium paraoxydans* in particular, could be used for reclamation of heavy metal-contaminated soil and wastewater.

Table 5. Maximum tolerance limit of DMAB* (*Microbacterium paraoxydans*) towards various heavy metals.

Heavy Metal	Maximum Tolerance Limit
Iron (Fe^{2+})	10 mM
Cobalt (Co^{2+})	3 mM
Zinc (Zn^{2+})	5 mM
Copper (Cu^{2+})	5 mM
Lead (Pb^{2+})	2 mM
Manganese (Mn^{2+})	5 mM
Nickel (Ni^{2+})	1 mM
Mercury (Hg^{2+})	0.5 mM
Cadmium (Cd^{2+})	1 mM

Table 6. Minimum inhibitory concentration of various heavy metals for DMAB* (*Microbacterium paraoxydans*).

Heavy Metal	Minimum Inhibitory Concentration (MIC)
Arsenite (As^{3+})	36.95 mM
Arsenate (As^{5+})	280.44 mM
Chromium (Cr^{3+})	63 mM
Chromium (Cr^{6+})	1 mM

3.5. Growth Patterns of DMAB in High Concentration of Arsenic (III) and Chromium (III)*

Almost identical growth patterns of DMAB* were observed in both the control medium (devoid of arsenic) and in presence of 10 mM arsenite (Figure 4). The results indicate that the bacterium could neutralize the toxic effects of such high concentrations of arsenic and maintain its normal growth pattern even in the presence of arsenic stress. This could be due to the utilization of arsenic as a substrate for deriving energy for driving metabolic processes, which might negate the toxic effects of the heavy metal. Another explanation could be the assimilation of arsenic into the bacterial biomass or there could be an interplay between assimilatory and dissimilatory processes [66–68]. In the presence of 10 mM chromium (III), DMAB* showed a prolonged lag phase. However, after the initial delay in the growth, the bacterial cells recovered and grew normally to reach the density of the bacterial culture grown in the control medium. The rapid transition to the log phase of the bacterium in the presence of arsenite could be explained by its natural adaptability towards high concentrations of arsenic, as it was exposed to prolonged arsenic stress in its natural habitat. In contrast, the bacterium showed a prolonged lag phase in presence of chromium (III) and later restored its rapid growth pattern in the log phase. The long lag phase might coincide with the time required for its adaptation towards a heavy metal Cr^{3+}, not predominant in its natural habitat.

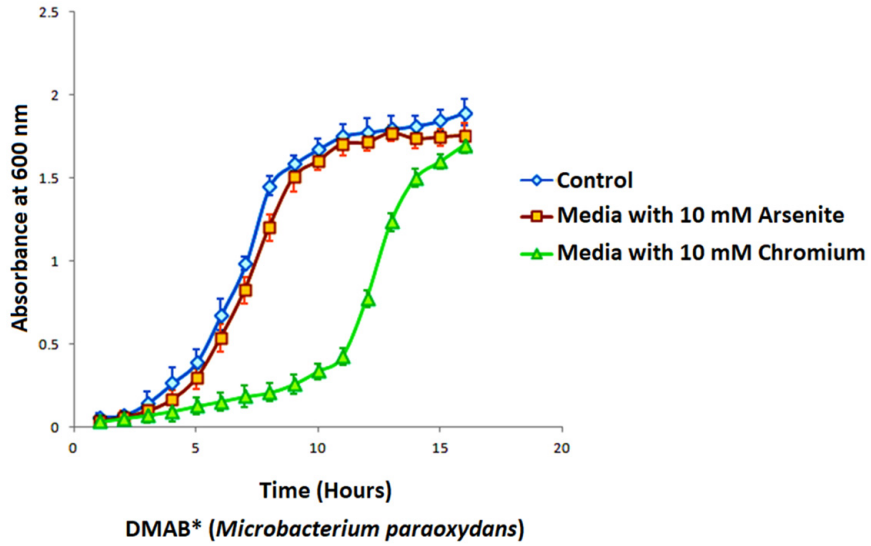

Figure 4. Growth patterns of the bacterial isolate. The bacterium was cultured in LB media supplemented with 10 mM arsenite (III) and 10 mM chromium (III). Medium without sodium arsenite served as control. The growth rate was checked by monitoring the optical density of the medium at 600 nm.

*3.6. Bioremediation of Arsenic Toxicity by DMAB**

It was observed by the standard SDDC method of arsenic estimation that DMAB* could bioremediate 30.8% and 35.2% of 1 mM arsenite over a period of 24 and 48 h,

respectively. A further increase in the arsenite concentration to 4 mM led to a slight reduction in the bioremediation efficiency (22.6% and 30.5% in 24 and 48 h, respectively) (Figure 5). Therefore, at high and very high concentrations of arsenite, the bacterium was capable of bioremediation of arsenic with significant efficiency. The underlying mechanism of the bioremediation process exhibited by the bacterium could be biotransformation, bioadsorption/bioaccumulation, or a combination of these processes. The bacterium might be involved in the enzymatic transformation (biotransformation) of arsenite to arsenate (the less toxic form of arsenic), thereby reducing the concentration of arsenite in the local environment. On the other hand, the accumulation of arsenite in the bacterial cytoplasm, either in free or conjugated form (bioaccumulation) or adsorption of arsenite on the bacterial membrane (bioadsorption), could be a part of the bacterial defense system, towards the tolerance of a high concentration of arsenite and subsequent reduction in its concentration. The bioremediation potential of this bacterium was lower than the arsenite removal efficiency of *Microbacterium* sp. strain 1S1, isolated from industrial wastewater of Sheikhupura, Pakistan. The inactivated biomass of the *Microbacterium* sp. strain 1S1 could remove 99% of 15 mM arsenite within 10 h of incubation [62]. *Microbacterium lacticum* isolated from municipal sewage could oxidize up to 50 mmoles/L of arsenite. The arsenite oxidation potential of *Microbacterium lacticum* could be applied for the removal of >99.9% arsenate using activated carbon in a biofilter, as suggested by Mokashi and Paknikar [69]. The physicochemical processes of arsenic elimination from groundwater or wastewater are expensive with considerable environmental costs. Therefore, the isolated strain of *Microbacterium paraoxydans* from this study could complement the existing filtration technologies by its bioremediation potential, as evident in the case of other species of *Microbacterium*. Hence, bioremediation used as a technology could turn out to be significant in the mitigation of arsenic toxicity [70,71].

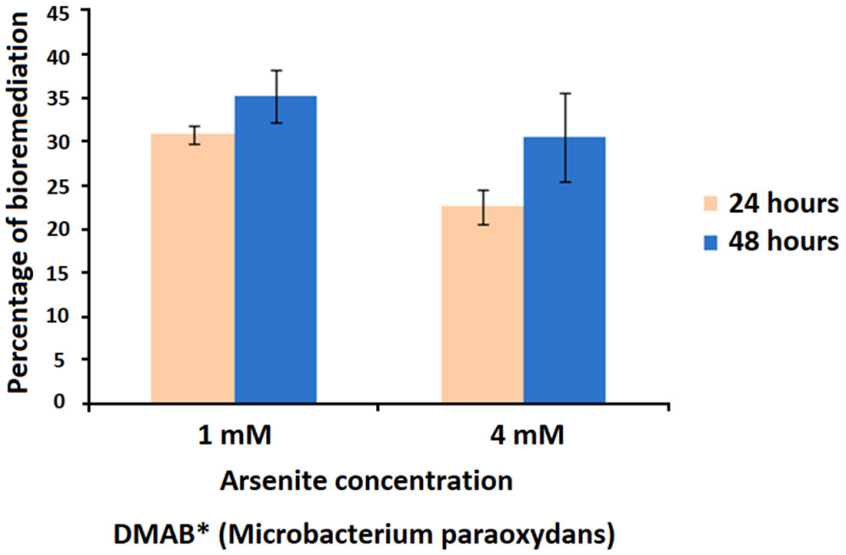

Figure 5. Arsenic bioremediation by arsenic hyper-tolerant strain of *Microbacterium paraoxydans*. The bar diagram represents percentage of bioremediation of arsenic by arsenic hyper-tolerant strain of *Microbacterium paraoxydans*, in varying concentrations (1 and 4 mM) of sodium arsenite at 37 °C for 24 and 48 h.

*3.7. Plant Growth-Promoting Properties Shown by DMAB**

IAA production, siderophore production, atmospheric nitrogen fixation and solubilization of insoluble potassium and phosphate, are some of the plant growth-promoting properties usually studied to predict the role of microbes in plant growth promotion. DMAB* could produce IAA and siderophore in a culture medium. Such plant growth-promoting properties are usually shown by soil bacteria, inhabiting the root and surrounding regions (rhizosphere) of plants. DMAB* could produce 0.77 µg/mL of IAA (per ml of media)

and 21.16% of siderophore. The bacterium was capable of fixing atmospheric nitrogen and solubilizing insoluble phosphates on agar plates containing Jensen and pikovskaya media, respectively. However, DMAB* could not solubilize insoluble potassium (Table 7). Different species of *Microbacterium* were found in the rhizosphere and categorized as a rhizobacteria. It is reported that *Microbacterium paraoxydans* is a well-known plant growth promoter. A strain of *Microbacterium paraoxydans* showing 99% similarity to *Microbacterium paraoxydans* strain M59 and *Microbacterium paraoxydans* strain M8S2B3 was reported to play a significant role in nodule formation in *Phaseolus* sp. [72]. Phosphate solubilizing *Microbacterium paraoxydans* was isolated from the root of Mexican husk tomato plants [73]. Hirsch et al., in their patent, claimed *Microbacterium paraoxydans* (NR025548) as one of the plant growth-promoting microorganisms and described the method of its application for plant growth promotion [74]. Cordovez da Cunha et al. also explained the method of applying *Microbacterium paraoxydans* as a novel plant growth-promoting bacteria in their patent [75]. *Microbacterium paraoxydans* showed antagonistic activity against *Fusarium* wilt disease of Chickpea [76]. Hence, the bacterium isolated in this study could be used as a potential biofertilizer after successful field trials for the enhancement of plant growth [77,78]. In addition to this, the bacterial isolate might also reduce the effect of arsenic toxicity in plants cultivated in arsenic-contaminated soil, by activating the arsenic defense mechanism [79]. An increase in arsenic tolerance might also lead to increased arsenic accumulation in plants, as reported by Alka et al. [80,81]. However, many plant growth-promoting bacteria are also known to reduce arsenic translocation, toxicity and accumulation in plants [82,83]. Therefore, the actual effect of this isolated strain on the plants grown in arsenic-contaminated soil could be a subject of future research. The ability of arsenic bioremediation and plant growth-promoting potential, explain the reason for inhabiting the arsenic-contaminated garden soil of the Bhagobangola I block by this bacterium.

Table 7. Plant growth-promoting properties of DMAB* (*Microbacterium paraoxydans*).

Plant growth Promoting Properties	Observation
# IAA production	+ (0.77 µg/mL)
* Siderophore production	+ (21.16%)
Atmospheric nitrogen fixation	+
Insoluble phosphate solubilization	+
Insoluble potassium solublization	−

* 48 h old culture was used for Siderophore estimation (having cfu 1.3×10^6/mL). # 72 h old culture was used for IAA estimation (having cfu 1.2×10^7/mL). +: positive growth/production. −: lack of growth/production.

4. Conclusions

Heavy metal hyper-tolerant bacteria are often found in heavy metal-contaminated soil and groundwater. In this study, an arsenic hyper-tolerant strain of *Microbacterium paraoxydans* has been isolated from the arsenic-contaminated garden soil of the Bhagobangola I block. The isolated strain shows tolerance towards other heavy metals such as chromium, copper, zinc and iron. The bacterium also exhibits potential plant growth-promoting properties such as IAA and siderophore production, atmospheric nitrogen fixation and insoluble potassium and phosphate solubilization. The arsenic bioremediation potential of the bacterium could be used for the mitigation of arsenic toxicity from soil and groundwater by its translation into a prominent technology which could be used in bio-filters. The plant growth-promoting properties of the bacterium allow it to be used as a potential biofertilizer. Therefore, *Microbacterium paraoxydans* could play the dual role of arsenic bioremediation from arsenic-contaminated soil and groundwater, and plant growth promotion when applied to the soil with a proper medium.

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References

1. Dangleben, N.L.; Skibola, C.F.; Smith, M.T. Arsenic immunotoxicity: A review. *Environ. Health* **2013**, *12*, 73. [CrossRef] [PubMed]
2. Samal, A.C.; Kar, S.; Maity, J.P.; Santra, S.C. Arsenicosis and its relationship with nutritional status in two arsenic affected areas of West Bengal, India. *J. Asian Earth Sci.* **2013**, *77*, 303–310. [CrossRef]
3. Guha Mazumder, D.N.; Haque, R.; Ghosh, N.; De, B.K.; Santra, A.; Chakraborty, D.; Smith, A.H. Arsenic levels in drinking water and the prevalence of skin lesions in West Bengal, India. *Int. J. Epidemiol.* **1998**, *27*, 871–877. [CrossRef]
4. Milton, A.H.; Hasan, Z.; Shahidullah, S.M.; Sharmin, S.; Jakariya, M.D.; Rahman, M.; Dear, K.; Smith, W. Association between nutritional status and arsenicosis due to chronic arsenic exposure in Bangladesh. *Int. J. Environ. Health Res.* **2004**, *4*, 99–108. [CrossRef] [PubMed]
5. Mondal, N.K.; Roy, P.; Das, B.; Data, J.K. Chronic arsenic toxicity and its relation with nutritional status: A case study in Purbasthali-II, Burdwan, west Bengal. *Int. J. Environ. Sci.* **2011**, *2*, 1103–1118. [CrossRef]
6. Maharjan, M.; Watanabe, C.; Ahmad, S.A.; Umezaki, M.; Ohtsuka, R. Mutual interaction between nutritional status and chronic arsenic toxicity due to groundwater contamination in an area of Terai, lowland Nepal. *J. Epidemiol. Community Health* **2007**, *61*, 389–394. [CrossRef] [PubMed]
7. Rahman, M.; Vahter, M.; Sohel, N.; Yunus, M.; Wahed, M.A.; Streatfield, P.K.; Ekstrom, E.C.; Persson, L.A. Arsenic exposure and age and sex-specific risk for skin lesions: A population-based case-referent study in Bangladesh. *Environ. Health Perspect.* **2006**, *114*, 1847–1852. [CrossRef]
8. Paul, S.; Majumdar, S.; Giri, A.K. Genetic susceptibility to arsenic-induced skin lesions and health effects: A review. *Genes Environ.* **2015**, *37*, 23. [CrossRef] [PubMed]
9. Shen, S.; Li, X.F.; Cullen, W.R.; Weinfeld, M.; Le, X.C. Arsenic Binding to Proteins. *Chem. Rev.* **2013**, *113*, 7769–7792. [CrossRef]
10. Islam, K.; Qian, W.; Yu, H.J.; Chao, W.; Hua, N. Metabolism, toxicity and anticancer activities of Arsenic compounds. *Oncotarget* **2017**, *8*, 23905–23926. [CrossRef]
11. Rahman, M.M.; Sengupta, M.K.; Ahamed, S.; Lodh, D.; Das, B.; Hossain, M.A.; Nayak, B.; Mukherjee, A.; Chakraborti, D.; Mukherjee, S.C.; et al. Murshidabad-One of the Nine Groundwater Arsenic-Affected Districts of West Bengal, India. Part I: Magnitude of Contamination and Population at Risk. *Clin. Toxicol.* **2005**, *43*, 823–834. [CrossRef] [PubMed]
12. SOES. *Groundwater Arsenic Contamination in West Bengal-India (20 Years Study)*; SOES: Kolkata, India, 2006.
13. Shamim, S. Biosorption of Heavy Metals, Biosorption, Jan Derco and Branislav Vrana, IntechOpen. 2018. Available online: https://www.intechopen.com/chapters/58112 (accessed on 10 October 2022). [CrossRef]
14. Katz, S.A.; Salem, H. The biological and environmental chemistry of chromium. In *The Toxicology of Chromium and Its Compound*; VCH Publishers Inc.: New York, NY, USA, 1994. [CrossRef]
15. Brooks, R.R. *Plants That Hyperaccumulate Heavy Metals: Their Role in Phytoremediation, Microbiology, Archaeology, Mineral Exploration and Phytomining*; University Press: Cambridge, MA, USA; Publisher CAB International: Wallingford, UK, 1998; 80p, ISBN 9780851991566. Available online: https://www.cabi.org/bookshop/book/9780851992365/ (accessed on 10 October 2022).
16. Xu, S.; Xu, R.; Nan, Z.; Chen, P. Bioadsorption of Arsenic from aqueous solution by the extremophilic bacterium *Acidithiobacillus ferrooxidans* DLC-5. *Biocatal. Biotransform.* **2018**, *37*, 35–43. [CrossRef]
17. Mandal, D.; Biswas, S.; Seal, S.; Mandal, R.; Das, S.; Basu, A. Arsenic toxicity and its clinical manifestations in Murshidabad district with some potential remedial measures. In *Microbes and Microbial Biotechnology for Green Remediation*; Elsevier: Amsterdam, The Netherlands, 2022.
18. Sharma, I. Bioremediation Techniques for Polluted Environment: Concept, Advantages, Limitations, and Prospects. In *Trace Metals in the Environment—New Approaches and Recent Advances*; Murillo-Tovar, M.A., Saldarriaga-Noreña, H., Saeid, A., Eds.; IntechOpen: London, UK, 2019. [CrossRef]

19. Santana, M.M.; Gonzalez, J.M. High temperature microbial activity in upper soil layers. *FEMS Microbiol. Lett.* **2015**, *362*, fnv182. [CrossRef] [PubMed]
20. Portillo, M.C.; Santana, M.; Gonzalez, J.M. Presence and potential role of thermophilic bacteria in temperate terrestrial environments. *Naturwissenschaften* **2012**, *99*, 43–53. [CrossRef] [PubMed]
21. Manzoni, S.; Schimel, J.P.; Porporato, A. Responses of soil microbial communities to water stress:results from meta-analysis. *Ecology* **2012**, *93*, 930–938. [CrossRef]
22. Biederman, J.A.; Scott, R.L.; Goulden, M.L.; Vargas, R.; Litvak, M.E.; Kolb, T.E.; Yepez, E.A.; Oechel, W.C.; Blanken, P.D.; Bell, T.W.; et al. Terrestrial carbon balance in a drier world: The effects of water availability in southwestern North America. *Glob. Chang. Biol.* **2016**, *22*, 1867–1879. [CrossRef] [PubMed]
23. Grady, E.N.; MacDonald, J.; Liu, L.; Richman, A.; Yuan, Z.-C. Current knowledge and perspectives of *Paenibacillus*: A review. *Microb. Cell Fact.* **2016**, *15*, 203. [CrossRef]
24. Das, H.K. Azotobacters as biofertilizer. *Adv. Appl. Microbiol.* **2019**, *108*, 1–43. [CrossRef]
25. do Amaral, F.P.; Tuleski, T.R.; Pankievicz, V.C.S.; Melnyk, R.A.; Arkin, A.P.; Griffitts, J.; Tadra-Sfeir, M.Z.; de Souza, E.M.; Deutschbauer, A.; Monteiro, R.A.; et al. Diverse Bacterial Genes Modulate Plant Root Association by Beneficial Bacteria. *mBio* **2020**, *11*, 6. [CrossRef]
26. Bhattacharya, P.; Chatterjee, D.; Nath, B.; Jana, J.; Jacks, G.; Vahter, M. High arsenic groundwater: Mobilization, metabolism and mitigation—An overview in the Bengal Delta Plain. *Mol. Cell Biochem.* **2003**, *253*, 347–355. [CrossRef]
27. Chatterjee, D.; Halder, D.; Majumder, S.; Biswas, A.; Nath, B.; Bhattacharya, P.; Bhowmick, S.; Mukherjee-Goswami, A.; Saha, D.; Hazra, R.; et al. Assessment of arsenic exposure from groundwater and rice in Bengal Delta Region, West Bengal, India. *Water Res.* **2010**, *44*, 5803–5812. [CrossRef] [PubMed]
28. Halder, D.; Bhowmick, S.; Biswas, A.; Chatterjee, D.; Nriagu, J.; Mazumder, D.N.G.; Šlejkovec, Z.; Jacks, G.; Bhattacharya, P. Risk of arsenic in exposure from drinking water and dietary components: Implications for risk management in rural Bengal. *Environ. Sci. Technol.* **2013**, *47*, 1120–1127. [CrossRef] [PubMed]
29. Santra, S.C.; Samal, A.C.; Bhattacharya, P.; Banerjee, S.; Biswas, A.; Majumdar, J. Arsenic in Foodchain and Community Health Risk: A Study in Gangetic West Bengal. *Procedia Environ. Sci.* **2013**, *18*, 2–13. [CrossRef]
30. Pelczar, M.J.; Bard, R.C.; Burnett, G.W.; Conn, H.J.; Demoss, R.D.; Euans, E.E.; Weiss, F.A.; Jennison, M.W.; Meckee, A.P.; Riker, A.J.; et al. *Manual of Microbiological Methods. Society of American Bacteriology*; McGraw Hill Book Company: New York, NY, USA, 1957; p. 315. [CrossRef]
31. Brown, A.E.; Benson, H.J. *Benson's Microbiological Applications. Laboratory Manual in General Microbiology, Short Version*, 10th ed.; The McGraw Hill Companies: New York, NY, USA, 2007; p. 50.
32. Jukes, T.H.; Cantor, C.R. Evolution of Protein Molecules. In *Mammalian Protein Metabolism*; Munro, H.N., Ed.; Academic Press: New York, NY, USA, 1969; pp. 21–132. [CrossRef]
33. Saitou, N.; Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **1987**, *4*, 406–425. [CrossRef] [PubMed]
34. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [CrossRef]
35. EFSA European Food Safety Authority. Guidance on the assessment of bacterial susceptibility to antimicrobial of human and veterinary importance. *EFSA J.* **2012**, *10*, 2740.
36. Sher, S.; Sultan, S.; Rehman, A. Characterization of multiple metal resistant *Bacillus licheniformis* and its potential use in arsenic contaminated industrial wastewater. *Appl. Water Sci.* **2021**, *11*, 69. [CrossRef]
37. Roy, P.; Mondal, N.K.; Das, B.; Das, K. Arsenic contamination in groundwater: A statistical modelling. *J. Urban Environ. Eng.* **2013**, *7*, 24–29. [CrossRef]
38. Jensen, H.L. Nitrogen fixation in leguminous plants I. General characters of root-nodule bacteria isolated from species of *Medicago* and *Trifolium* in Australia. *Proc. Linn. Soc. New South Wales* **1942**, *67*, 98–108.
39. Pikovskaya, R.I. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Microbiology* **1948**, *17*, 362–370.
40. Ouertani, R.; Ouertani, A.; Mahjoubi, M.; Bousselmi, Y.; Najjari, A.; Cherif, H.; Chamkhi, A.; Mosbah, A.; Khdhira, H.; Sghaier, H.; et al. New Plant Growth-Promoting, Chromium-Detoxifying Microbacterium Species Isolated from a Tannery Wastewater: Performance and Genomic Insights. *Front. Bioeng. Biotechnol.* **2020**, *8*, 521. [CrossRef] [PubMed]
41. Sun, F.; Ou, Q.; Wang, N.; Guo, Z.; Ou, Y.; Li, N.; Peng, C. Isolation and identification of potassium-solubilizing bacteria from *Mikania micrantha* rhizospheric soil and their effect on *M. micrantha* plants. *Glob. Ecol. Conserv.* **2020**, *23*, e01141. [CrossRef]
42. Rahman, A.; Sitepu, I.R.; Tang, S.Y.; Hashidoko, Y. Salkowski's reagent as a primary screening index for functionalities of rhizobacteria isolated from wild dipterocarpsapling growing naturally on medium-strongly acedictropical peatsoil. *Biosci. Biotechnol. Biochem.* **2010**, *74*, 2202–2208. [CrossRef]
43. Payne, S.M. Detection, isolation and characterization of siderophore. *Methods Enzymol.* **1994**, *235*, 329–344. [CrossRef] [PubMed]
44. Mandal, D.; Sonar, R.; Saha, I.; Ahmed, S.; Basu, A. Isolation and identification of arsenicresistant bacteria: A tool for bioremediation of arsenic toxicity. *Int. J. Environ. Sci. Technol.* **2021**, *19*, 9883–9900. [CrossRef]
45. Prieto, D.M.; González, J.C.; Barral, M. Arsenic Mobility in As-Containing Soils from Geogenic Origin: Fractionation and Leachability. *J. Chem.* **2018**, *2018*, 7328203.

46. Singh, A. Chemistry of Arsenic in Groundwater of Ganges–Brahmaputra River Basin. *Curr. Sci.* **2006**, *91*, 599–606. Available online: http://www.jstor.org/stable/24094363 (accessed on 10 October 2022).
47. Chakraborty, M.; Mukherjee, A.; Ahmed, K.M. A Review of Groundwater Arsenic in the Bengal Basin, Bangladesh and India: From Source to Sink. *Curr. Pollut. Rep.* **2015**, *1*, 220–247. [CrossRef]
48. Buss, S.N.; Starlin, R.; Iwen, P.C. Bacteremia caused by *Microbacterium binotii* in a patient with sickle cell anemia. *J.. Clin. Microbiol.* **2014**, *52*, 379–381. [CrossRef]
49. Qian, F.; An, L.; Wang, M.; Li, C.; Li, X. Isolation and characterization of a xanthan-degrading *Microbacterium* sp. strain XT11 from garden soil. *J. Appl. Microbiol.* **2007**, *102*, 1362–1371. [CrossRef]
50. Jung, Y.; Lee, S.; Kim, N.; Yoon, H.; Jeong, G.; Lee, B.; Park, H.; Hur, M. *Microbacterium caeni* sp. nov., a novel species isolated from sludge in Yeosu, Korea. *Res. Sq.* **2022**, PPR473869. [CrossRef]
51. Hadjadj, L.; Rathored, J.; Keita, M.B.; Michelle, C.; Levasseur, A.; Raoult, D.; Fournier, P.; Rolain, J.; Bittar, F. Non contiguous-finished genome sequence and description of *Microbacterium gorillae* sp. nov. *Stand Genom. Sci.* **2016**, *11*, 32. [CrossRef]
52. González Henao, S.; Ghneim-Herrera, T. Heavy Metals in Soils and the Remediation Potential of Bacteria Associated With the Plant Microbiome. *Front. Environ. Sci.* **2021**, *9*, 604216. [CrossRef]
53. Kaushik, P.; Rawat, N.; Mathur, M.; Raghuvanshi, P.; Bhatnagar, P.; Swarnkar, H.; Flora, S. Arsenic Hyper-tolerance in Four *Microbacterium* Species Isolated from Soil Contaminated with Textile Effluent. *Toxicol. Int.* **2012**, *19*, 188–194. [CrossRef]
54. Mohd Bahari, Z.; Ibrahim, Z.; Jaafar, J.; Shahir, S. Draft Genome Sequence of Arsenic-Resistant *Microbacterium* sp. Strain SZ1 Isolated from Arsenic-Bearing Gold Ores. *Genome Announc.* **2017**, *5*, e01183-17. [CrossRef]
55. Bermanec, V.; Paradžik, T.; Kazazić, S.P.; Venter, C.; Hrenović, J.; Vujaklija, D.; Duran, R.; Boev, I.; Boev, B. Novel arsenic hyper-resistant bacteria from an extreme environment, Crven Dol mine, Allchar, North Macedonia. *J. Hazard Mater.* **2021**, *402*, 123437. [CrossRef]
56. Ben Fekih, I.; Zhang, C.; Li, Y.P.; Zhao, Y.; Alwathnani, H.A.; Saquib, Q.; Rensing, C.; Cervantes, C. Distribution of Arsenic Resistance Genes in Prokaryotes. *Front. Microbiol.* **2018**, *9*, 2473. [CrossRef]
57. Koechler, S.; Cleiss-Arnold, J.; Proux, C.; Sismeiro, O.; Dillies, M.; Goulhen-Chollet, F.; Hommais, F.; Lièvremont, D.; Arsène-Ploetze, F.; Coppée, J.-Y.; et al. Multiple controls affect arsenite oxidase gene expression in *Herminiimonas arsenicoxydans*. *BMC Microbiol.* **2010**, *10*, 53. [CrossRef]
58. Chang, J.S.; Yoon, I.H.; Lee, J.H.; Kim, K.-R.; An, J.; Kim, K.-W. Arsenic detoxification potential of *aox* genes in arsenite-oxidizing bacteria isolated from natural and constructed wetlands in the Republic of Korea. *Environ. Geochem. Health* **2010**, *32*, 95–105. [CrossRef]
59. Achour-Rokbani, A.; Cordi, A.; Poupin, P.; Bauda, P.; Billard, P. Characterization of the ars gene cluster from extremely arsenic-resistant *Microbacterium* sp. strain A33. *Appl. Environ. Microbiol.* **2010**, *76*, 948–955. [CrossRef]
60. Chowdhury, R.; Sen, A.K.; Karak, P.; Chatterjee, R.; Giri, A.K.; Chaudhuri, K. Isolation and characterization of an arsenic-resistant bacterium from a bore-well in West Bengal, India. *Ann. Microbiol.* **2009**, *59*, 253–258. [CrossRef]
61. Dey, U.; Chatterjee, S.; Mondal, N.K. Isolation and characterization of arsenic-resistant bacteria and possible application in bioremediation. *Biotechnol. Rep.* **2016**, *10*, 1–7. [CrossRef]
62. Sher, S.; Hussain, S.; Cheema, M.T.; Hussain, A.; Rehman, A. Efficient removal potential of *Microbacterium* sp. strain 1S1 against arsenite isolated from polluted environment. *J. King Saud Univ.-Sci.* **2022**, *34*, 102066. [CrossRef]
63. Maizel, D.; Balverdi, P.; Rosen, B.; Sales, A.M.; Ferrero, M.A. Arsenic-hypertolerant and arsenic-reducing bacteria isolated from wells in Tucumán, Argentina. *Can. J. Microbiol.* **2018**, *64*, 876–886. [CrossRef]
64. Waranusantigul, P.; Lee, H.; Kruatrachue, M.; Pokethitiyook, P.; Auesukaree, C. Isolation and characterization of lead-tolerant Ochrobactrum intermedium and its role in enhancing lead accumulation by Eucalyptus camaldulensis. *Chemosphere* **2011**, *85*, 584–590. [CrossRef] [PubMed]
65. Singh, V.; Mishra, V. Microbial removal of Cr (VI) by a new bacterial strain isolated from the site contaminated with coal mine effluents. *J. Environ. Chem. Eng.* **2021**, *9*, 106279. [CrossRef]
66. Stolz, J.F.; Oremland, R.S. Bacterial respiration of arsenic and selenium. *FEMS Microbiol. Rev.* **1999**, *23*, 615–627. [CrossRef]
67. Oremland, R.S.; Stolz, J.F. The ecology of arsenic. *Science* **2003**, *300*, 939–944. [CrossRef]
68. Stolz, J.F.; Basu, P.; Santini, J.M.; Oremland, R.S. Arsenic and selenium in microbial metabolism. *Annu. Rev. Microbiol.* **2006**, *60*, 107–130. [CrossRef]
69. Mokashi, S.A.; Paknikar, K.M. Arsenic (III) oxidizing Microbacterium lacticum and its use in the treatment of arsenic contaminated groundwater. *Lett. Appl. Microbiol.* **2002**, *34*, 258–262. [CrossRef]
70. Zhu, Y.G.; Rosen, B.P. Perspectives for genetic engineering for the photoremediation of arsenic contaminated environments: From imagination to reality? *Curr. Opin. Biotechnol.* **2009**, *20*, 220–224. [CrossRef] [PubMed]
71. Chandraprabha, M.N.; Natarajan, K.A. Mechanism of arsenic tolerance and bioremoval of arsenic by Acidithiobacilus ferrooxidans. *J. Biochem. Tech.* **2011**, *3*, 257–265. Available online: https://jbiochemtech.com/en/article/mechanism-of-arsenic-tolerance-and-bioremoval-of-arsenic-by-acidithiobacilus-ferrooxidans (accessed on 10 October 2022).
72. Tekdal, D.; Akça, I.; Küçükrecep, A.; Çetiner, S.; Hatipoğlu, R. Characterization of root-associated bacterial species of Leklek variety of common bean (*Phaseolus* sp.). *Rom. Biotechnol. Lett.* **2021**, *26*, 3008–3013. [CrossRef]

73. Hernández-Pacheco, C.E.; Orozco-Mosqueda, M.D.C.; Flores, A.; Valencia-Cantero, E.; Santoyo, G. Tissue-specific diversity of bacterial endophytes in Mexican husk tomato plants (*Physalis ixocarpa* Brot. ex Horm.), and screening for their multiple plant growth-promoting activities. *Curr. Res. Microb. Sci.* **2021**, *2*, 100028. [CrossRef] [PubMed]
74. Hirsch, A.M.; Monica, S.; Kaplan, D.; Sheva, B. Plant growth-promoting microorganisms and methods of use thereof. U.S. Patent 10212943B2, 26 February 2019.
75. da Cunha, V.C.; Hanssen, I.; De Ceuster, T.J.J.; Carrion-Bravo, V.J.; Raaijmakers, J.M. Novel plant-growth promoting bacteria and the use thereof. Patent No. EP3311669A1, 25 April 2018.
76. Khanna, A.; Raj, K.; Kumar, P.; Wati, L. Antagonistic and growth-promoting potential of multifarious bacterial endophytes against Fusarium wilt of chickpea. *Egypt. J. Biol. Pest Control.* **2022**, *32*, 1–9. [CrossRef]
77. Song, Y.; Han, J.; Hu, Y. Diversity analysis of culturable bacteria in the root of tree peony (*Paeonia ostii*). In Plant Growth-Promoting Rhizobacteria (PGPR) for Sustainable Agriculture, Proceedings of the 2nd Asian PGPR Conference, Beijing, China, 21–24 August 2011.
78. Anandaraj, M.; Bini, Y.K. Potential of PGPR application for seed spices with special reference to coriander and fenugreek in India. In Plant Growth-Promoting Rhizobacteria (PGPR) for Sustainable Agriculture, Proceedings of the 2nd Asian PGPR Conference, Beijing, China, 21–24 August 2011.
79. Soto, J.; Ortiz, J.; Herrera, H.; Fuentes, A.; Almonacid, L.; Charles, T.C.; Arriagada, C. Enhanced Arsenic Tolerance in *Triticum aestivum* Inoculated with Arsenic-Resistant and Plant Growth Promoter Microorganisms from a Heavy Metal-Polluted Soil. *Microorganisms* **2019**, *7*, 348. [CrossRef] [PubMed]
80. Alka, S.; Shahir, S.; Ibrahim, N.; Rahmad, N.; Haliba, N.; Abd Manan, F. Histological and proteome analyses of *Microbacterium foliorum*-mediated decrease in arsenic toxicity in *Melastoma malabathricum*. *3 Biotech* **2021**, *11*, 336. [CrossRef]
81. Alka, S.; Shahir, S.; Ibrahim, N.; Vo, D.N.; Abd Manan, F. Assessment of plant growth promotion properties and impact of *Microbacterium foliorum* for arsenic removal in Melastoma malabathricum. *Bioremediation J.* **2022**, *26*, 1–12. [CrossRef]
82. Sun, Y.; Wang, X.; Liu, Y.; Duan, K.; Xia, Y.; Cai, Q.; Lou, L. Long term application of plant growth-promoting bacterium improved grain weight and reduced arsenic accumulation in rice grain: A comparison of 10 bacteria. *Chemosphere* **2022**, *303 Pt 1*, 135016. [CrossRef]
83. Thongnok, S.; Siripornadulsil, W.; Siripornadulsil, S. AsIII-oxidizing and Cd-tolerant plant growth-promoting bacteria synergistically reduce arsenic translocation, toxicity and accumulation in KDML105 rice. *Environ. Exp. Bot.* **2021**, *192*, 104660. [CrossRef]