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Article

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

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Abstract: The soil and groundwater of Bhagobangola I block of Murshidabad district, West Bengal, India is severely arsenic contaminated. A bacterium was isolated from the garden soil of 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 & V) and chromium (III) hyper-tolerant bacterium. The growth pattern of this bacterium does not show much alteration in presence of 10 mM arsenic (III) and chromium (III), emphasizing its resistance to these heavy metals. Scanning electron microscopy depicted the size of this bacterium to be ~1.45 µm. 16S rDNA sequencing followed by subsequent phylogenetic analysis established the identity of this bacterium as *Microbacterium paraoxydans*. The bacterium is capable of bioremediation of arsenic and showed 30.8% & 22.6%, and 35.2% and 30.5% of bioremediation over a period of 24 and 48 hours in 1 mM and 4 mM arsenite, respectively. *Microbacterium paraoxydans* also exhibit plant growth promoting properties like 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 of arsenic toxicity in soil and groundwater but also for plant growth promotion by using it as a biofertilizer.

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

1. Introduction

At present, arsenic toxicity of soil and groundwater is a global problem. Arsenic exists in nature in both inorganic and organic forms. The inorganic forms of arsenic include arsenite (+3), arsenate (+5), arsenic (0) and arsenide (-3). The organic forms of arsenic include monomethyl arsenic acid (MMA), dimethyl arsenic acid (DMA), arsenobetaine and arsenocholine. Arsenite has high solubility, mobility and bioavailability, and it is 100 times more toxic compared to 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 geogenic origin. The anthropogenic sources of arsenic include pesticides, fungicide, weedicide, wood preservative, etc. Nine district of West Bengal have arsenic contaminated soil and groundwater. Murshidabad district is worst affected by arsenic toxicity. Bhagobangola I & II, Lalgola, Beldanga I, Jalangi, Domkal and

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Hariharpara blocks of Murshidabad district on the eastern bank of Bhagirathi river show much higher arsenic contamination than the blocks on the western bank. The problem of arsenic toxicity in 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 resistance to arsenic. Arsenite oxidase operon aox*ABCD*, 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 transportation and sequestration of arsenic in different compartments of 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 arsenic related 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, large concentration of Chromium (III) in the body may cause health hazards including lung cancer [14]. Bioremediation is a well established technology used for mitigation of heavy metal toxicity and xenobiotic stress from the environment by application of various biological agents. The remediation process could be mediated by bacteria, plants (phytoremediation), fungi (rhizoremediation), etc. Biotransformation (transformation of the pollutant into 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 [15-17].

Application of bacteria as biofertilizer has turned out to be an effective as well as ecofriendly way, to reduce the use of chemical fertilizers. The promotion of plant growth is usually accomplished by production of siderophores, indole-3-acetic acid (IAA), solubilization of phosphate, zinc, potassium, biological nitrogen fixation and production of ACC deaminase by the bacteria. These biofertilizers could play an important role in maintaining sustainable agriculture and industrial biotechnology [18]. Microorganism like azotobacter participate significantly in fixation of atmospheric nitrogen (by aerobic process), production of plant hormones, solubilization of insoluble phosphate and reduction of harmful and deleterious effects of phytopathogens and xenobiotics, and thereby, resulting in better production of crops like wheat, barley, rice, oat [19]. The plant growth promoting bacteria recognize their suitable host plant and colonize the plant roots to increase the growth of plants either directly (by nitrogen fixation, plant hormones 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 had been proved to be one of the best ways of enhancing plant growth, extensive research is needed, mainly in agricultural fields in presence of multiple variable abiotic and biotic factors, in order to decipher the best way of using such bacteria as biofertilizer [20].

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 [21-24]. However, the presence of different microbial communities and their potential role in reclamation of arsenic contaminated groundwater and soil needs further investigation, particularly in 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. In this study, we have isolated a heavy metal hyper-tolerant bacterium from the garden soil of highly arsenic contaminated Bhagobangola I block of Murshidabad district. The bacterium is resistant to very

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high concentration of arsenite, arsenate and chromium (III). The isolate is capable of arsenic bioremediation and possesses plant growth promoting properties like nitrogen fixation, phosphate solubilisation, IAA and siderophore production. Therefore, potentially, this bacterium could be used as biofertilizers to increase crop yield, in general. Also, its heavy metal hyper-tolerance property could be applied for designing a bio-filter, in order to reduce the concentration of arsenic in groundwater and wastewater. Such heavy metal hyper-tolerant plant growth promoting bacteria might also enhance crop yield in heavy metal contaminated agricultural fields, particularly in underdeveloped blocks of Murshidabad district.

2. Materials and methods

2.1. Selection of the study area

Groundwater and soil samples of Bhagobangola I block of Murshidabad district is highly arsenic contaminated [11-12]. Garden soil samples were collected from 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

100 mg of the garden soil collected from Bhagobangola I block was dissolved in 100 ml double distilled water to achieve a concentration of 1 mg/ml. Soil and Water Testing Kit (ORLAB) was used for estimation of pH and iron. Arsenic and phosphate in the garden soil were estimated using Arsenic Testing Kit (Merck) and spectrophotometrically using ammonium molybdate, respectively.

2.3. Isolation and characterization of arsenic hyper-tolerant bacteria

Garden soil sample collected from 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 concentration 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 like glucose, sucrose, fructose, lactose, mannitol, inositol and mannose was also checked by following the standard protocol [25]. The sensitivity of the isolate was tested for commonly used antibiotics. They were cultured in LB media supplemented individually with 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 hours at 37º C. The bacterial growth was checked after the incubation period [26].

2.4. Scanning electron microscope imaging of the isolated bacterium

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

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2.5.16S rDNA sequencing and phylogenetic analysis for identification of the isolated bacterium

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. Fragment of 16S rDNA gene was amplified by 27F and 1492R 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 PCR amplicon was carried out with 27F and 1492R primers, using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of 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 maximum identity score, highest query coverage and lowest E value, 15 sequences were selected and aligned with rDNA sequence of DMAB*, using multiple sequence alignment software program Clustal W. 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 [27-29].

2.6. Determination of heavy metal resistance by the isolated bacterium

The bacterial isolates were tested for their resistance to heavy metals like Cu^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Hg^{2+}, Fe^{2+}, Zn^{2+}, Mn^{2+} and Pb^{2+}. 500 mM stock solutions of $CuSO_4.5H_2O$, $CoCl_2.6H_2O$, $MnSO_4.H_2O$, $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$ and 100 mM stock solution of $CdCl_2.H_2O$, $PbCl_2$, $NiCl_2.6H_2O$ 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 them on LB media amended with variable concentration of the heavy metals starting from 0.5 mM. The bacterial growth was detected after incubation for 24 hours at 37 ºC. The bacterial growth was tested in LB medium by turbidometric method and in LB-Agar medium by formation of bacterial colony.

2.7. Determination of minimum inhibitory concentration of arsenic (III and V) and chromium (III and VI) for the isolated bacterium

For determination of Minimum Inhibitory Concentration (MIC), the bacterium was inoculated in Luria Broth (LB) media supplemented individually, with increasing concentration 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 LB medium by turbidometric method.

2.8. Study of the growth curve of isolated bacterium

The bacterium was inoculated into LB media, individually containing 10 mM arsenite and 10 mM chromium (III). LB medium without any 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 arsenic hyper-tolerant bacterium in LB media containing two different concentration of sodium arsenite, 1mM and 4 mM. The control culture was inoculated in LB medium without any arsenic stress. All these sets were incubated under stirring condition (160 rpm) at 37ºC for 24 hours. After incubation, the bacterial cells were separated from media by centrifugation at 10000 rpm for 5 minutes. Arsenic concentration of the media after bioremediation was estimated by SDDC method [30].

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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 soil, was tested by culturing the bacterium on Jensen's agar medium (a nitrogen deficient medium). The potential of the bacterium to solubilize insoluble phosphorus and potassium was tested on Pikovskaya agar (with insoluble source of phosphate) and Aleksandrow agar (with insoluble source of potassium) media, respectively. The bacterial growth was assessed by formation of bacterial colonies on the respective agar plates. The formation of 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 colour of the medium from green to blue, indicates towards increase in pH due to the formation of ammonia, as a result of atmospheric nitrogen fixation by the bacterium.

2.11. Indole-3-acetic acid production by the isolated bacterium

Pure and fresh bacterial inoculum was added to LB medium containing 0.5 mg/L of tryptophan. The culture was incubated at 28 ^{o}C with continuous shaking at 125 rpm for 48 hours. 2 ml of culture was centrifuged at 15000 rpm for 1 minute. 1 ml supernatant was mixed with 2ml Salkowski's solution. The mixture was incubated at room temperature in dark condition. Absorbance of the pink color developed 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 [31].

2.12. Siderophore production by the isolated bacterium

10 µl of fresh culture (10^{8} CFU/ml) was spotted on chrome azurol sulphonate (CAS) agar plate. The plate was incubated at 28 ^{o}C for 72 hours. Change in color of the CAS agar medium from blue to orange indicated towards siderophore production. For quantitative assay, 0.5 ml of culture supernatant was mixed with 0.5 ml CAS reagent and incubated for 5 minutes. Absorbance at 630 nm was measured against a reference of uninoculated broth. Percent siderophore unit (psu) in the culture supernatant was calculated using the formula given below.

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 [32].

3. Results and discussion

Mahishasthali village of Bhagobangola I block has soil and groundwater contaminated with high concentration of arsenic. Garden soil sample contaminated with arsenic was collected from Mahishasthali village and used for downstream work. In our previous study, we have reported about two arsenic resistant bacteria isolated from agricultural soil of Asanpara village [11-12, 33]. In this study, an arsenic hyper-tolerant bacterium was isolated from the garden soil of 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 Murshidabad district to report 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 to Bhagobangola I railway station **(Figure 1)**. The pH of the soil is in slightly basic range. The phosphate, although present in low amount (0.02 mg/L), might allow higher solubility, mobility and leachability of arsenic. The presence of iron (0.03 mg/L) in the soil allows 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 (0.1 ppm) concentration in the garden soil is a matter of concern, as arsenic might get incorporated into crops/vegetables grown

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in the soil and get biomagnified in the food chain. However, the presence of another toxic element fluoride could not be detected in the soil **(Table 1)** [34-36].

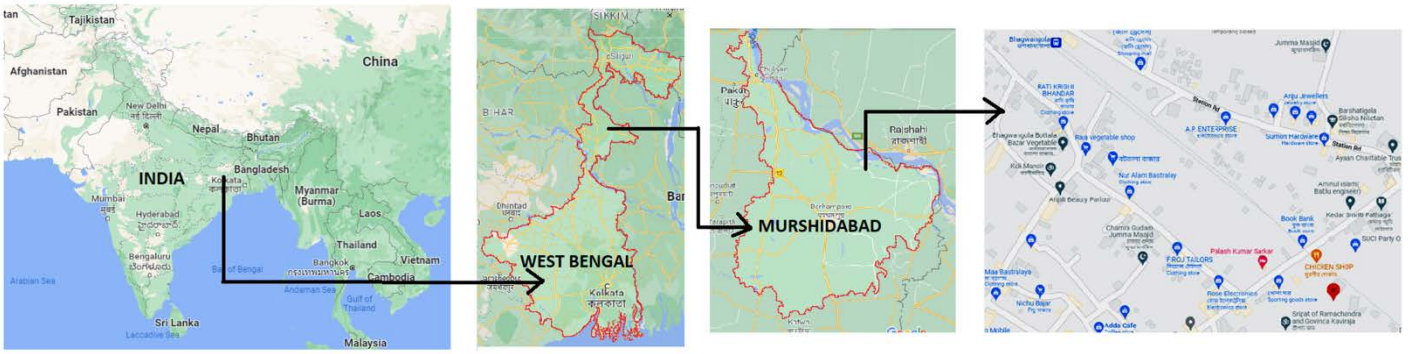

Figure 1. Map of study area in Mahishasthali village of Bhagobangola I block of Murshidabad district

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

Arsenic hyper-tolerant bacterium was isolated from the garden soil by serially diluting the soil sample and subsequent plating in LB agar plates, with increasing concentration of sodium arsenite. On LB agar plates, the isolated bacterium formed round, yellow pigmented mucoid colonies of moderate size **(Figure 2, Table 2)**. The bacterium DMAB* is gram positive and acid fast negative in nature. Scanning electron microscopy further confirmed that the bacterium is having a rod shape with a length of ~1.45 μm **(Figure 3, Table 2)**. The aforementioned findings corroborates with the characteristics of different species of *Microbacterium* characterized by Qian et al., Jung et al. and Hadjadj et al. [37-39]. The isolated bacterium was sensitive to recommended dose of common antibiotics like 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 like glucose, fructose, sucrose and mannitol but not lactose, mannose and inositol **(Table 4).**

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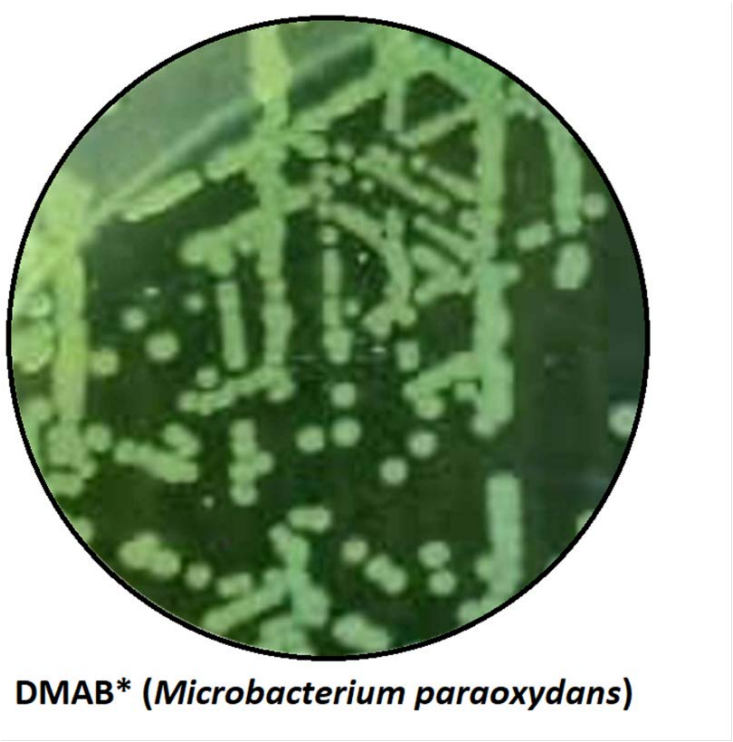

Figure 2. Isolation of arsenic hyper-tolerant bacterium from arsenic contaminated garden soil. Colonies of DMAB* cultured in LB-agar medium supplemented with 7.70 mM (1000 ppm) sodium arsenite.

Table 2. Morphological profile of the isolated strain.

Colony morphological profile	
Form	Round
Colour	Yellow pigmented
Texture	Mucoid
Size	Moderate
Microscopic morphological profile	
Gram's nature	Gram positive
Acid fast	Negative
Shape	Rod
Size	1.45 µM

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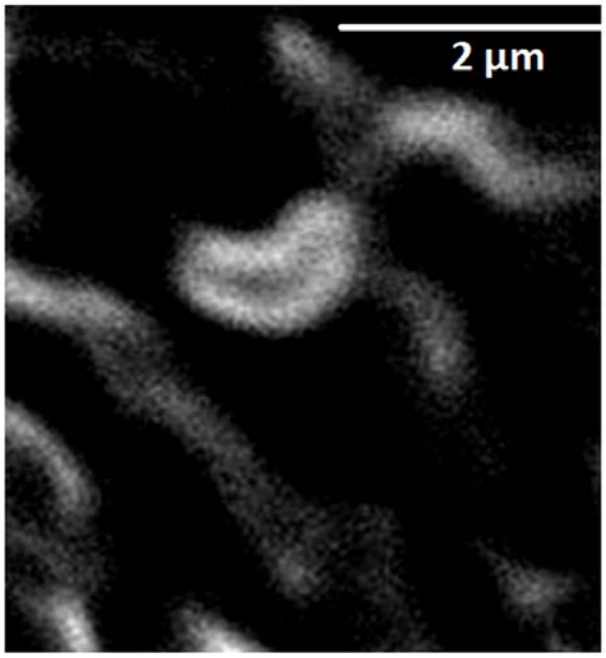

Figure 3. Scanning electron microscope (SEM) image of DMAB* (*Microbacterium paraoxydans*). SEM analysis showed DMAB* is a rod shaped bacteria with length ~1.45 µm.

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

Antimicrobial agent	Concentration (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 isolated bacterium.

Carbohydrates	Fermentation ability
Glucose	+
Fructose	+
Mannose	-
Sucrose	+
Lactose	-
Mannitol	+
Inositol	-

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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* like strain 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 highest identity (99.60%) and query coverage (100%) with the rDNA sequence of DMAB*. In genome assembly, chromosome I of *Microbacterium paraoxydans* strain DSM 15019, showed 99.46% identity and 100 % query coverage with rDNA sequence of DMAB* **(Figure 4)**. Different members of the genus *Microbacterium* were reported as arsenic tolerant bacteria in India and throughout the world. Highly arsenic resistant *Microbacterium paraoxydans* 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. They also showed resistance to zinc, chromium, selenium and other heavy metals and sensitivity towards mercury, cadmium, etc [40]. In addition to this, arsenic resistant *Microbacterium* sp. strain SZ1 was isolated from arsenic bearing gold ores of Malaysia [41]. *Microbacterium spp*. isolated from Creven Dol Mine, Allchar, North Macedonia- survived in presence of 209 mM arsenite and 564 mM arsenate [42].

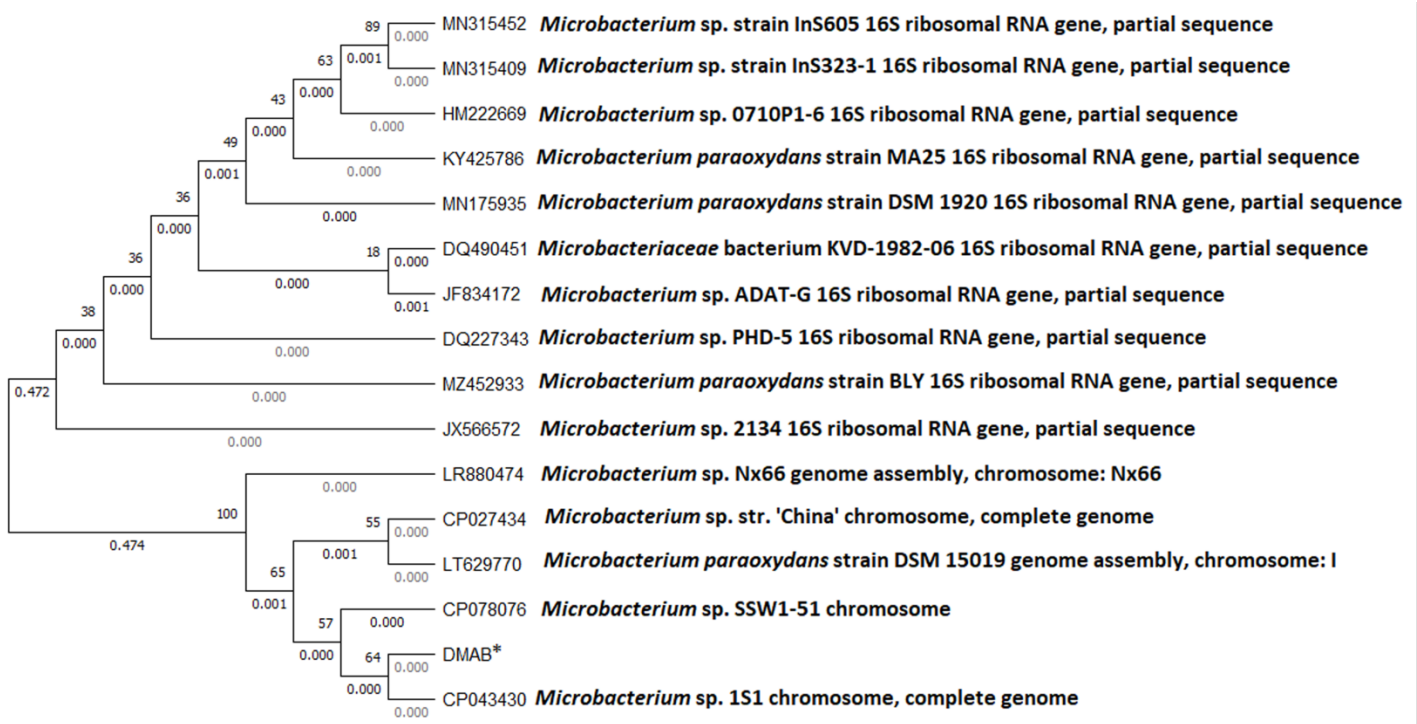

Figure 4. Phylogenetic tree of bacterial isolate DMAB* (*Microbacterium paraoxydans*)

3.4. Heavy metal tolerance and susceptibility of the isolated bacterium

The isolated *Microbacterium paraoxydans* could tolerate moderately high concentration (5 mM) of some heavy metals like zinc (Zn^{2+}), copper (Cu^{2+}) and manganese (Mn^{2+}). It could also survive in high concentration of iron (Fe^{2+}) (10 mM). However, the isolate was susceptible towards low concentration 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 arsenic tolerance

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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 concentration up to 4500 ppm and arsenite concentration up to 550 ppm [43-44]. Lead tolerant *Microbacterium paraoxydans* BN-2 was isolated from the rhizosphere of *E. camaldulensis*, from lead contaminated soils of Bo Ngam mine, Thailand [45]. *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% of removal efficiency in presence of 50 mg/L of chromium (Cr^{6+}) [46]. Therefore, the genus *Microbacterium* consists of numerous heavy metal resistant species, which were reported to be resistant to multiple heavy metals like arsenic, chromium, lead, etc. Along with this, 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*) to 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+})	1mM

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* was obtained in both the control medium (devoid of arsenic) as well as in presence of 10 mM arsenite **(Figure 5)**. This indicated that the bacterium could neutralize the toxic effects of such high concentration 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 could 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 [47-49]. 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.

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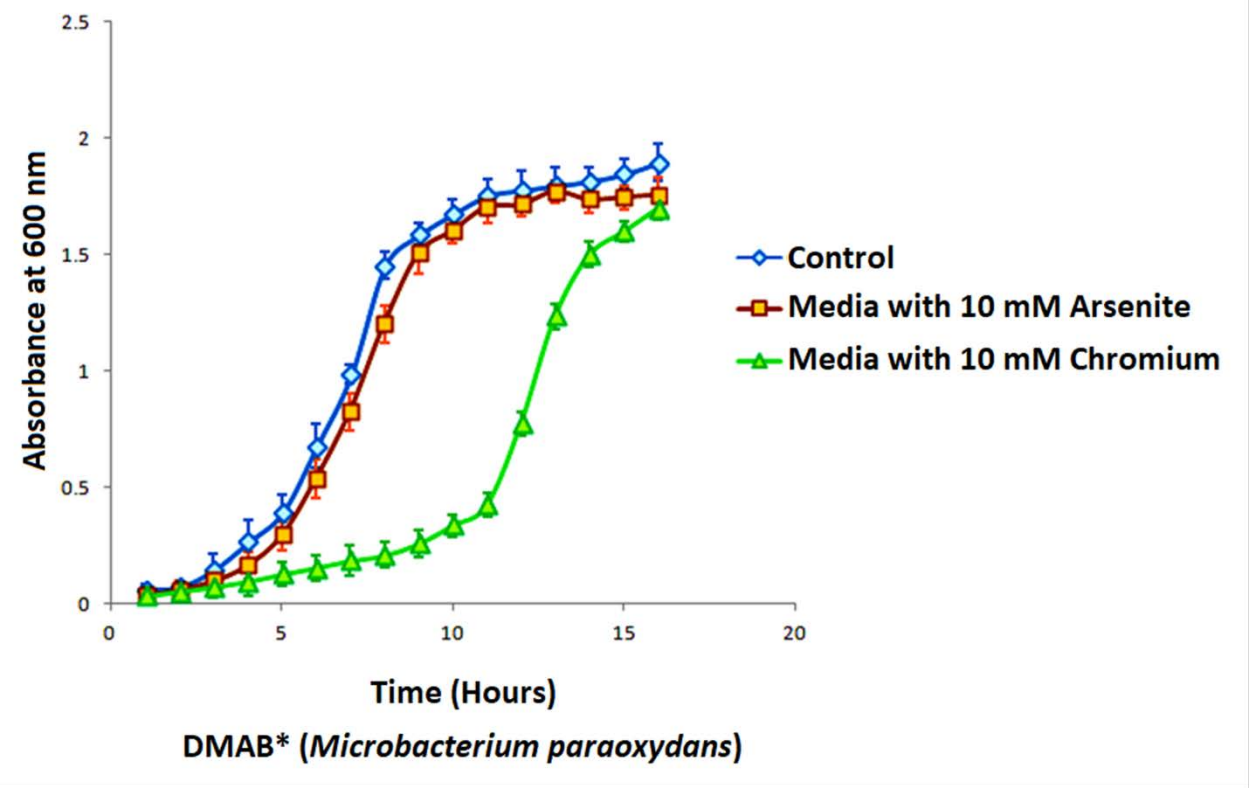

Figure 5. Growth patterns of the bacterial isolate. The bacterium was cultured in LB media supplemented with 10 mM arsenite (III) and 10 mM chromium (III). Medium without sodium arsenite served as control. The growth rate was checked by monitoring the optical density of the medium at 600 nm.

*3.6. Bioremediation of arsenic toxicity by DMAB**

It was observed by 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 hours, respectively. Further increase in the arsenite concentration to 4 mM led to slight reduction in the bioremediation efficiency (22.6 % and 30.5 % in 24 and 48 hours, respectively) **(Figure 6)**. Therefore, at high and very high concentration of arsenite, the bacterium was capable of bioremediation of arsenic with a 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 physicochemical processes of arsenic elimination from groundwater or wastewater are expensive with considerable environmental cost. Therefore, the isolated strain of *Microbacterium paraoxydans* from this study could complement the existing filtration technologies by its bioremediation potential. Hence, bioremediation used as a technology, could turn out to be significant in mitigation of arsenic toxicity [50-51].

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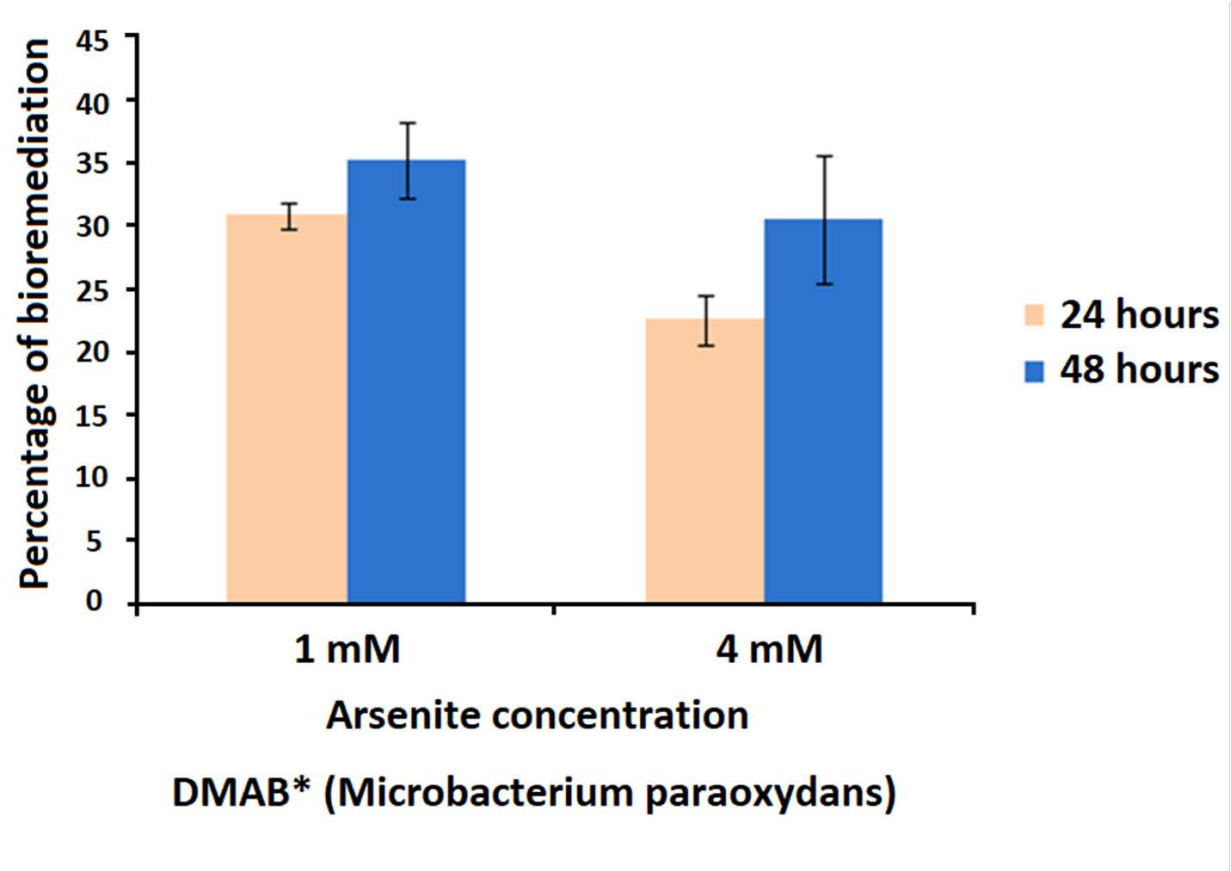

Figure 6. Arsenic bioremediation by arsenic hyper-tolerant bacteria. The bar diagram represents percentage of bioremediation of arsenic by arsenic resistant bacteria in varying concentration (1 and 4 mM) of sodium arsenite at 37 ºC for 24 and 48 hours.

*3.7. Plant growth promoting properties shown by DMAB**

DMAB* could produce IAA and siderophore in culture medium. Such plant growth promoting properties are usually shown by soil bacteria, inhabiting the root and surrounding regions (rhizobacteria) of plants. DMAB* could produce 0.77 µg/ml of IAA (per ml of media) and 21.16 % of siderophore. Also, 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. Also, it is reported that *Microbacterium paraoxydans* is a well known plant growth promoter. Hence, this bacterium could be used as a biofertilizer for enhancement of fertility of the soil [52-53]. Therefore, ability of arsenic bioremediation and plant growth promotion explain the reason for inhabiting the arsenic contaminated garden soil of 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 hr old culture for Siderophore estimation (having cfu 1.3 x 10^6 /ml).
*72 hr old culture for IAA estimation (having cfu 1.2 x 10^7/ml).
* +: positive growth/production.
*–: lack of growth/production

4. Conclusion

Heavy metal hyper-tolerant bacteria with arsenic bioremediation potential could be used for mitigation of arsenic toxicity from soil and groundwater. Such bioremediation potential is turning into a prominent technology to be used in bio-filters. Also, the plant growth promoting potential of the bacterium allows it to be used as a biofertilizer. Therefore, *Microbacterium paraoxydans* could play the dual role of inhibition of biomagnification of arsenic in the food chain and increasing the crop yield when applied to the soil with a proper medium.

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Declaration

Authors' contributions: **DM, AB** and **DC** conceived the idea and design of research. **DM, MA, SKD, SM, DC** and **AB** conducted the experiments. **DM, MA, SKD, SM, DC** and **AB** analyzed the data. **DM, AB** and **DC** were involved in manuscript preparation. All authors read and approved the manuscript.

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