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Research Article

Identification and Evaluation of Arsenic Tolerant Bacteria for Arsenic Mitigation in Contaminated Soil

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Abstract

A total of seventy two bacterial strains were isolated from different areas of Bangladesh to find and evaluate Arsenic (As) tolerant bacteria for mitigation of arsenic contamination and other biotechnological application. Strain colonies were circular, groove and flat in shapes and size ranged from 0.3 mm to 5.8 mm with white, off white, orange, yellow color. Among the strains, the strain TAN-8 was able to grow in high concentrations (28 mM) of arsenic. The highest arsenic tolerant strain TAN-8 showed maximum growth at 37°C and at pH-7 after 34 h of inoculation. The strain TAN-8 was arsenic metabolizing bacteria since it produced violet color in silver nitrate test and suggesting that this strain uses arsenic for its own growth and development. The ERIC-PCR fingerprinting of arsenic tolerant TAN-8 strain showed seven different DNA bands with 400 bp to > 1500bp long. Sequencing and phylogenetic analysis of 16S rRNA gene confirmed that the strain TAN-8 was *Klebsiella pneumoniae* (100%). The strain TAN-8 was capable of effective metabolism of arsenic and survives in high arsenic condition along with high temperature and pH. Thus, the strain TAN-8 could be used for mitigation of arsenic contaminated environment and to reduce arsenic uptake by crops grown in Arsenic contaminated soil.

Keywords: Arsenic Tolerant Bacteria; Mitigation of Arsenic, Arsenic Uptake by Crops

Abbreviations

As: Arsenic; BINA: Bangladesh Institute of Nuclear Agriculture; ATSDR: Agency for Toxic Substances and Disease Registry; LB: Luria Bertani; $AgNO_3$: Silver Nitrate; cm: Centimeter; ML: Maximum Likelihood; K2P: Kimura Two-Parameter; ERIC: Enterobacterial Repetitive Intergenic Consensus; bp: Base Pairs

Introduction

Heavy or toxic metals are trace metals with a density at least five times higher than water and are detrimental to human health [1]. Ecosystems are exposed to heavy metals heavily due to industrial and mining activities, and they also occur during fuel production from waste materials. These result in unnatural and harmful presence of heavy metals in environment because they form stable molecules and do not breakdown organically [2]. The ATSDR [3] (agency for toxic substances and disease registry) reported that arsenic is one the most harmful element for the ecosystem since it deposits heavily in lipid tissues of organism, which in turn accumulate in even heavier proportion in the upper level of the trophic chain. Sodium arsenite exposure can occur by inhalation or skin absorption which causes skin irritation, burns, itching, stomach pain, nausea, vomiting, diarrhea, convulsions, carcinogenic and teratogenic effects etc. [4]. Significant level of Arsenic exposure may affect nervous system leading to a number of conditions ranging from weakness, poor coordination, or "pins and needles" sensations to the extent of paralysis and even death [5,6]. Arsenic toxicity in crop plants caused by transfer of arsenic from contaminated soil is of great concern because of its potential health hazards [7] because about 30% of the total arsenic ingestion is caused by arsenic polluted rice and other food sources [8]. Breakdown of heavy metals by microorganisms is known as bioremediation (https://study.com/academy/lesson/types-of-bioremediation.html). Microorganisms can interact with metals via many mechanisms such as biosorption, biotransformation, bioaccumulation, biomineralisation, microbially-enhanced chemisorption of metals, biodegradation of chelating agents and bioleaching. Some of which may be used as the basis of potential bioremediation strategies [9]. Bacteria could be used to mitigate arsenic from soil and plants. For example, Mallick et al. [10] used two arsenic resistant bacteria (Kocuria flava and Bacillus vietnamensis) for reducing arsenic accumulation in rice. By 84.8% and 82.2%, respectively. Wang et al. [11] showed that Populus deltoides LH05-17 (Poplar plant) is an efficient arsenic accumulator but high concentration of arsenic reduces its growth. Agrobacterium radiobacter D14 was used with Populus deltoides LH05-17 and it helped to tolerate even 300 mg /kg arsenic in soil and showed 54% arsenic removal. Therefore, bioremediation with microbes could have a remarkable impact in solution that issue. Sequencing of the 16S rRNA gene along with REP-PCR and ERIC-PCR are powerful techniques in bacterial taxonomy and their identification [12]. Isolation and identification of As resistant bacteria could be an avenue for microbial remediation of this heavy metal. Thus, the objective of the present study was to isolate and identify arsenic tolerant bacteria and to evaluate their arsenic tolerance level, their morphology and physiology for their further application in mitigation of arsenic contaminated environment and to reduce arsenic uptake by rice.

Materials and Methods

Collection of Samples

Tannery effluents and municipal solid wastes samples were

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collected from Hazaribagh tannery industrial area, Dhaka and bypass area, Mymensing, Bangladesh. A total of 22 samples were collected from those locations. Among them, 14 were soil and 8 were water samples. Water and soil samples were collected in sterilized plastic bottles and bags and then transported to Bangladesh Institute of Nuclear Agriculture (BINA) and preserved at 4°C in fridge until bacterial isolation process was initiated [13].

Isolation of heavy metal resistant bacteria

For the isolation of heavy metal resistant bacteria, at first Luria Bertani (LB) agar plates (Peptone 10.00 g/L, yeast extract, 5.00 g/L, NaCl 5.00 g/L, and agar 18-20.00 g/L: pH 7.00) were prepared with different concentration of arsenic. 10 g of soil and 10 mL of water from each sample were added to 90 mL sterile water and a dilution series up to 10^{-5} was prepared according to Azad et al. [14] to isolate desired bacteria. 10 µL aliquots from each dilution (10^{-2} to 10^{-5}) were poured on Luria Bertani agar plates that contained 4 mM of arsenic. Control plates were also prepared without including arsenic into the LB media. Sterilized spreader was used for spreading the diluted aliquot by rotating the plate. Inoculated plates were incubated at 37°C for 2-3 days. Meanwhile bacterial colonies appeared. Individual colonies with distinct morphologies were picked and streaked on Luria Bertani agar medium containing 6 mM of arsenic to get pure single colonies [13]. Distinct single colonies were grown in liquid LB medium. All the cultures were stored in 50% glycerol at -80°C for further study.

Evaluation of Arsenic tolerance

72 isolates were tested for their resistance to arsenic. LB-agar plates were prepared with different concentrations of arsenic. The starting concentration of arsenic in this test was 7 mM which was gradually increased to 42 mM. Freshly cultured bacteria were streaked on arsenic containing LB plate at 37°C for 4 days and the growth response of bacteria was observed in different concentrations of arsenic. Arsenic tolerance was assessed according to standard protocol of European Food Safety Authority [15].

Determination of growth curves

For the determination of growth curves, maximum arsenic tolerant strain TAN-8 was grown with and without arsenic stress in 100 mL Luria Bertani broth prepared in conical flask. Experiments were performed in triplicates. Medium was inoculated with 10 µL bacterial cultures and incubated for 44 h at 37°C in shaking incubator. One (1) mL of bacterial sample was drawn in a cuvette with the help of micropipette in laminar air flow every two hours. Optical density of TAN-8 broth was determined at 600 nm using spectrophotometer (Eppendorf Biophotometer: Eppendof AG 2233). A growth curve was plotted by taking optical density on Y-axis and incubation time on X-axis [16].

Determination of optimum pH and temperature

About 20 mL of sterilized Luria Bertani broth was taken in 50 mL flasks. The pH range of medium was adjusted from 4.0 to 10.0. Each pH was taken in triplicates with and without arsenic stress (20 µg/L). They were then inoculated with 10 µL of fresh culture of each bacterial isolates and incubated at 37°C in shaking incubator. After 24 h optical density was noted in spectrophotometer at 600 nm. A graph was plotted between optical density along Y-axis and pH along X-axis. The optimum pH of the strain was determined by graph. For the temperature measurement, sterilized 50 mL Luria Bertani broth was prepared in 100 mL conical flask. After inoculation with 10 µL culture, bacteria were grown in flask with and without arsenic stress (20 µg/L) that were kept at different temperatures ranging from 25°C to 40°C for 24 h. Experiments were conducted in triplicate. Optical density of each strain was noted and graphs were plotted taking optimum density along Y- axis and temperature along X- axis [16].

Verification of arsenic transforming ability of arsenic tolerant strain

Silver nitrate ($AgNO_3$) method was used to verify the transforming ability of bacterial strain. Arsenic tolerant bacterial strains were streaked on Luria Bertani agar plate containing 10 mg/L of arsenic. Plates were filled with 0.1 M $AgNO_3$ solution and then incubated at 37°C for 48 h [17].

Seedling emergence test

The bacterial strain (TAN-8) was grown in LB broth. Exponentially growing cells' broth cultures were used for inoculation. Rice seeds (advanced line CC-2 and advanced line -1) were surface sterilized using 70% ethanol in Erlenmeyer flask for 1 min and were treated with 3% sodium hypochlorite for 3 min followed by six to seven times washing with sterile water. After that, the seeds were soaked in TAN-8 bacterial broth for one hour. Seeds soaked in sterile LB broth (Without TAN-8) were treated as control. After soaking, the air-dried seeds were used for germination in sterilized Petri dishes containing agar 1% and kept at 28°C for 10 days [18].

Germination trait parameters

Germination rate was calculated according to the method by Krishnaswamy and Seshu [19]. Measurement of root and shoot length was taken after 10 days of seed setting. Measurement of root and shoot length was carried out as follows- five seedlings were randomly selected from each Petri dish and measured with a measuring scale and expressed in centimeters (cm) [20].

DNA isolation, ERIC-PCR and sequencing of 16S rRNA gene

Bacterial culture was grown at 37°C overnight in LB medium and the DNA was isolated following the protocol described by Chen and Kuo [21]. Extracted DNA was dissolved in TE buffer and the concentration was measured by UV-spectrophotometry. The PCR amplifications were performed with about 100 ng of template DNA. ERIC-PCR primers (forward: ATGTAAGCTCCTGGGGAT and reverse AAGTAAGTGACTGGGGGTGAGC) described by De Bruijn [22] were used. The PCR product was visualized on 3% (w/v) agarose gel. The conditions for ERIC–PCR were at 95°C for 5 min for Initial denaturation. Thirty PCR cycles containing denaturation at 94°C for 30 s, annealing at 52°C for 1 min, and elongation at 65°C for 8 min were used. The final elongation was at 65°C for 16 min.

For species identification, the 16S rRNA gene was amplified by using the primers described by Weisburg et al. [23]. The forward (AGAGTTTGATCCTGGCTCAG) and reverse (AAGGAGGTGATTCCAGCC) primers were used to amplify 16S rRNA gene. DNA amplification was performed in a bio-red gradient thermal cycler, PTC-200. The PCR product was visualized on 1.5% (w/v) agarose gel. The PCR conditions for the amplification of 16S

Figure 1: Growth of As tolerant bacteria (TAN-8).

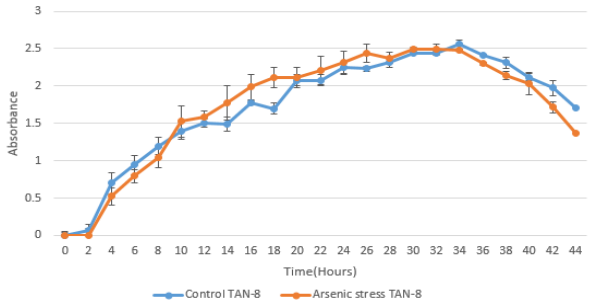

Figure 2: Effect of arsenic on the growth of the strain TAN-8.

Figure 3: Effect of pH on the growth of the strain TAN-8.

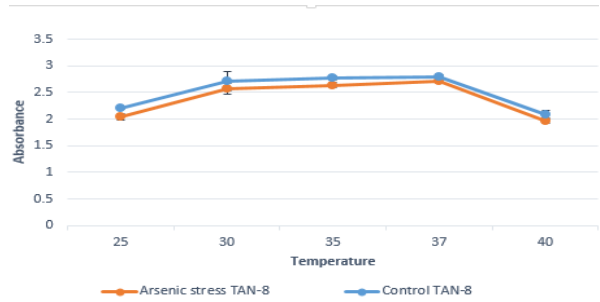

Figure 4: Effect of Temperature on the growth of the strain TAN-8.

rRNA gene were: initial denaturation at 95°C for 5 min. Thirty PCR cycles containing denaturation at 95°C for 1 min, annealing at 57°C for 1 min, and elongation at 72°C for 1.5 min were used. The final elongation was at 72°C for 15 min. Amplified PCR product was purified using PCR clean up system from Promega, USA. Sequencing was performed using an ABI 3730 automated capillary sequencer (Applied Biosystems) with the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit version 3.1 by 1st BASE (Singapore). To confirm the observed sequences quality, both strands were sequenced from arsenic tolerant strain.

Phylogenetic Analyses

The sequences were aligned with Bio Edit [24]. Phylogenetic trees were reconstructed using the Neighbor-Joining (NJ) algorithm [25] and Maximum Likelihood Methods (ML) in MEGA version-7 [26] using the Kimura two-parameter (K2P) model [27] and GTR model [28]. Bootstrap support for each node was evaluated with 1000 replicates.

Result

During the period of study, a total of 72 bacterial strains were isolated from 22 soil and water samples from selected locations of Bangladesh. All strains had different types of colonies. Bacterial colony shapes were circular, some of them were groove and the remaining was flat. Colony size of the isolates was ranged from 0.3 to 5.8 mm. Most of the colony colors were white and off white, whereas the rest of the colonies were orange, yellow and transparent (Table 1).

Arsenic Tolerance Level

The present study focused on the effects of as on bacterial growth to find the maximum arsenic tolerant bacteria to use them for managing the arsenic pollutant from arsenic contaminated environment. It was found that the growth of bacterial isolates decreased with increasing concentration of As (Table 2). Seventy-two TAN (1- 29, 31, 32, 34, 35, 36, 37, 39, 40, 42, 43, 44, 45, 47, 48, 49, 50, 54-58, 60, 64, 65, 66, 68-76, 79-87) bacterial strains showed resistance to arsenic at 4 mM. The starting concentration of heavy metal (As) in this test was 4 mM, which was gradually increased to 28 mM. Maximum resistance level (28 mM) was observed in the strain TAN-8 (Figure 1). Based on arsenic resistance level, pH tolerance and temperature tolerance, TAN-8 was selected for further studies.

Growth curves of arsenic tolerant TAN-8 strain with and without arsenic stress

The lag phase of isolated TAN-8 strain during arsenic stress condition was more extended compared to control. The lag phase,

Table 1: Morphological characteristic of bacterial isolates.

Isolate no.	Colony Shape	Colony Size (mm)	Colony Color
TAN-(1-13, 18-22, 24-29, 31, 35-37, 39, 40, 43, 47)	Circular	0.3- 5.3	Orange, Off white, Transparent, White, Yellow
TAN-(54, 55, 58, 60, 64, 65, 73, 74, 76, 80-82, 85)	Circular	0.3- 5.3	Orange, Off white, Transparent, White, Yellow
TAN-(14, 15, 17, 23, 32, 34, 66, 79, 86)	Groove	0.6- 4.2	White, Off white
TAN-(42, 44, 45, 48-50, 56, 57, 68-72, 75, 83, 84, 87)	Flat	0.9- 5.8	White, Off white

Table 2: Heavy metal (As) tolerance profile of selected bacterial isolates.

Bacterial Strain	Maximum arsenic tolerance (mM)
TAN- (31,32,34,35,42,43,45,47)	7
TAN -(3,17,19,21,24-26,28,54-58,60,64,69-72,75,79-81,84-87)	8
TAN- (5)	12
TAN -(14,50,68)	13
TAN -(4,11,12,18,27,39,40, 44, 48,49)	17
TAN -(65,66,73,74)	18
TAN -(1,6,7,9,29,36,37)	25
TAN -(2,10,13,15,16,20,22,23,76,82,83)	27
TAN -(8)	28

which normally occurs in the beginning of inoculation, is slightly influenced in the arsenic stress medium because bacteria have to utilize extra energy to repair the cell wall that gets damage by toxic effect of arsenic. This leaves bacteria with less energy that allows only a little growth when bacteria have to adapt to the new environmental conditions [29].

The lag phase, which was extended in the arsenic-stress medium normally, occurs in the beginning of inoculation because the arsenic has toxic effect on the cell wall of bacteria and damage it. So, bacteria expand lot of energy to repair it. So, little growth occurs as the bacteria are becoming 'acclimatized' to the new environmental conditions [29]. In exponential phase, bacterial growth was slower in arsenic stress medium compared to control medium up to 8 h, then bacterial growth was higher from 10 to 28 h in arsenic stress medium compared to control medium. In stationary phase, bacterial growth was similar at both medium from 32 to 34 h. Finally, death phase started after 36 h and bacterial death rate was higher in arsenic stress medium than control medium (Figure 2).

Optimum pH and temperature for arsenic tolerant strain

To check the effect of pH, the bacterial strain was grown at a pH range of 4.0-10.0 with and without arsenic stress. The results showed that optimum pH in controled and stressed medium for TAN-8 growth was 7.0. TAN-8 could tolerate basic pH and was sensitive to acidic pH (Figure 3).

To determine the optimum temperature of arsenic tolerant bacterial isolate in controled and stressed condition, it was grown at different temperature ranges of 25-40°C. The optimum temperature for growth of TAN-8 was 37°C in both controled and stressed medium. The bacterial isolates are more sensitive to change in pH than temperature (Figure 4).

The maximum pH and optimum temperature in arsenic-stress medium were the same as in controled medium. However, the optical density of TAN-8 was slightly lower in all conditions mentioned above for stressed bacteria because arsenic act as a toxic substance and hinder the bacterial growth in earlier hours. Then after adjusting to the media environment bacteria start to grow [30].

Verification of arsenic transforming ability of arsenic tolerant strain

Transforming ability of bacterial isolate was monitored by silver nitrate ($AgNO_3$) method. The appearance of violet colour indicated the presence of arsenite which showed that our arsenic tolerant strain was arsenic metabolizing bacteria (Figure 5) [31].

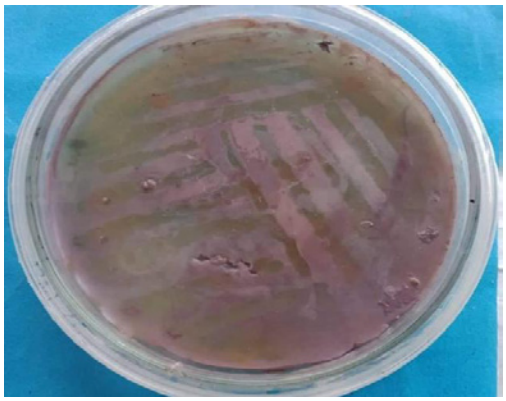

Figure 5: The presence of arsenic metabolizing bacterial strain TAN-8 on LB agar plate containing Silver nitrate.

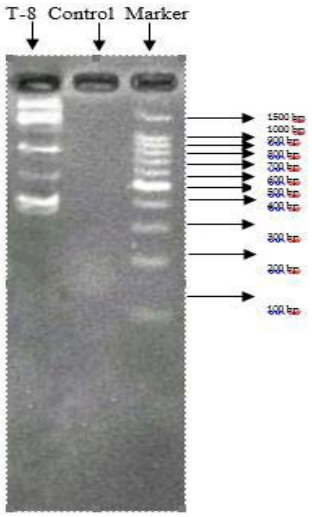

Figure 6: DNA fingerprinting of TAN-8 bacterial strain.

Effect of bacteria (TAN-8) on germination and growth of rice

After 10 days, a bunch of well-developed shoots axes with long and slender primary roots were observed. The maximum recorded

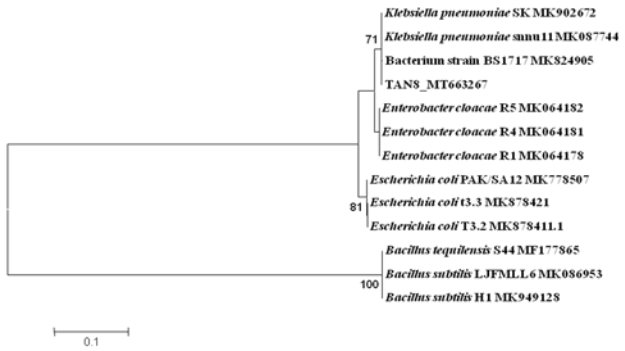

Figure 7: Maximum likelihood tree based on 16SrRNA gene partial sequences. Bootstrap values indicated when ≥70% (1000replicates).

length of root and shoot of advanced rice line CC-2 were 11.03 and 5.40 cm in TAN-8 inoculated seeds, whereas 11.20 and 5.34 cm were found in control. In rice, the highest length of root and shoot were found 9.98 and 4.80 cm in TAN-8 inoculated seeds, while 10.10 and 5.00 cm were recorded in control. Rice seed inoculation/priming suggested that, arsenic tolerant bacterial strain TAN-8 had neutral effects on root and shoot growth of rice.

Molecular Characterization

DNA fingerprinting of TAN-8 bacterial strain using ERIC-PCR

Enterobacterial Repetitive Intergenic Consensus (ERIC) sequences are widespread in the genome of bacteria and these elements are useful for fingerprinting genera, species, and strains of bacteria. This PCR is considered as powerful tool in bacterial taxonomy and may help in the determination of phylogenetic relationships [32, 22]. Thus, we use this PCR for fingerprinting our arsenic tolerant strain. We got seven DNA bands with the size of >400bp to greater than 1500 bp (Figure 6).

Sequencing of 16S rRNA gene and phylogenetic analysis

From TAN-8 bacterial strain 1397 base pairs (bp) sequence were obtained from the amplified and purified 16S rRNA gene PCR product. Obtained sequence was checked and aligned with Bio-Edit software [24]. A BLAST search with this sequence showed a high similarity (100%) to *Klebsiella pneumonia* strains. Bootstrap support for each node was evaluated with 1000 replicates. The Maximum Likelihood (ML) phylogenetic tree based on 16S rRNA revealed that the isolate TAN-8 belonged to the bacterial species *Klebsiella pneumoniae* (Figure 7).

Sequence

The following 16S rRNA gene sequence was obtained from arsenic tolerant TAN-8 bacterial strain. Obtained 16S-rRNA gene sequence was submitted to NCBI GeneBank and accession number is MT663267.

A G T C G A G C G G T A G C A C A G A G A G C T T G C T C T C G G G T G A C G A G C G G C G G A C G G G G T G A G T A A T G T C T G G G A A A C T G C C T G A T G G A G G G G G A T A A C TACTGGAAACGGTAGCTAATACCGCATAACGTCGCA A G A C C A A A G T G G G G G A C C T T C G G G C C T C A T G C C A T C A G A T G T G C C C A G A T G G G A T T A G C T A G T A G G T G G G G T A A C G G C T C A C C TAG GCGAC G A T C C C T A G C T G G T C T G A G A GGATGACCAGCCACA C T G G A A C TG A G A CAC G G T C C A GA C T CCT AC G G G A G GC A G C A G T G G G G A A T A T T G C A C A A TGGGCGCAAGCCTGA TGCAGCCATGCCGCGTGTGTGAAGA A G G C C T T C G G G T T G T A A A G C A C T T T C A G C G G G G A G G A A G G C G A T G A G G T T A A T A A C C T T G T C G A T T G A C G T T A C C C G C A G A A G A A G C A C C G G C T A A C T C C G T G C C A G C A G C C G C G G T A A T A C G G A G G G T G C A A G C G T T A A T C G G A A T T A C T G G G C G T A A A G C G C A C G C A G G C G G T C T G T C A A G T C G G A T G T G A A A T C C C C G G G C T C A A C CTGGGAACTGCATTCGAAACTGGCAGGCTAGAG T C T T G T A G A G G G G G G T A G A A T T C C A G G T G T A G C G G T G A A A T G C G T A G A G A T C T G G A G G A A T A C C G G T G G C G A A G G C G G C C C C C T G G A C A A A G A CTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGA T T A G A T A C C C T G G T A G T C C A C G C C G T A A A C G A T G T C G A T T T G G A G G T T G T G C C C T T G A G G C G T G G C T T C C G G AGCTAACGCGTTAAATCG A C C G C C T G G G G A G T A C G GC C G C A A GG T T A A A A C T C A A A T G A A T T G A C G G G G G CCCGCACAAG CGGTGGAGCATGT G G T T T A A T T C G A T G C A AC G C G A A G A A C C T T AC C T GG T C T T G A CA TCCACAGAACTTTCCAGAGATGG A T T G G T G C C T TCGGGAACTGTGA G A C A G G T G C T G C A T G G C T G T C G T C AGC T C G T G T T G T G A A A T G T T G G G T T A A G T C C C G C A A C G A G C G C A A C C C T T A T C C T T T G T T G C C A G C G G T TCG G C C G G G A A C T C A AAGG AG A CTGCCAGTGATAAACTG G A G G A A G G T G G G G A T G A C G T C A A G T C ATCATGGCCCTTACGACCAGGGCTACACAC T G C T A C A A T G G C A T A T A C A AA G A G A A G C G A C C T C G CG A G A G C A A G C G G A C C T CA T A A A G T A T G T C G T AG T CC G GA T T G G A G T C T GCA A C T C G A CT CC AT G A A GT C GGA AT C G C T A G T A A T C G T A GA TC A G A A T G C T A C G G T G AA TA C G T T C C C G G G G C C T T G T A C A C A CCG CC C G TC A CA CCATGGG AGTGGGTTGCAAA AGAAGTAGGTAGCTTAACC TTCGGGAGG

Discussion

This research work was conducted for the isolation and identification of heavy metal arsenic tolerant bacteria and evaluation of their arsenic tolerance level for further biotechnological application. About 95% of all microorganisms produce beneficial effects by increasing nutrients digestion and assimilation, preventing pathogens development and by improving environmental parameters [33]. A total of 72 bacterial strains were isolated in present study. Our bacterial strain, as well, showed beneficial effect by showing high tolerances to arsenic, pH and temperature etc. The maximum tolerance level of arsenic was observed at 28 mM by the strain TAN-8. By sequencing and phylogenetic analysis of 16S rRNA gene, we confirmed that this strain belongs to the species *Klebsiella pneumoniae*. Indigenous microbial strains of different taxonomic

traits may potentially have the capability to degrade harmful chemicals that cause environmental contamination [34]. In this context, we isolated arsenic resistant strains of *Klebsiella pneumoniae* from our environment. It was observed that growths of bacterial strains decreased with the increase of heavy metal concentrations which was similar to the previous findings [35,36]. The strain TAN-8 was found to tolerate arsenic 28 mM suggesting that this strain are tolerant to high concentration of arsenic. Most of the bacteria found in industrial wastewater are the members of genera *Bacillus, Dienococcus, Pseudomonous, Acidthiobacilus* and *Desulfitobacterium* and shown resistance against arsenic [37]. But present study found arsenic tolerant bacteria from industrial wastewater, which belongs to the genus *Klebsiella*. Our finding of *K. pneumoniae* as an arsenic tolerant bacteria is relatable to a previous study that observed this particular bacteria's ability to oxidize As (III) to As (V) and the presence of *ars* operon in *K. pneumoniae*, which can reduce As (V) to As (III) [16]. Further studies found that arsenic tolerant bacteria survive in arsenic stress condition by arsenic reducing capacity; but our arsenic tolerant strain showed metabolizing properties by showing violet color in silver nitrate ($AgNO_3$) test suggesting that it may use arsenic for its own growth and development. This bacterial strain could be used in microbial bioremediation of As from contaminated environments since a similar result was also found by researchers [38,16] and could be used as inoculant for reducing arsenic uptake by crops cultivated in Arsenic contaminated environments [10].

Conclusion

Due to continued unsustainable levels of human exploitation and certain natural phenomena, search for alternative techniques for treatment and management of arsenic contaminated wastewater and environment is essential. Arsenic bioremediation approach using microbes has attracted much attention because they are environment friendly, safe and economical. The microbes are the main machinery of this technology. We found one bacterial strain which has the maximum tolerance level (28 mM) in arsenic. Heavy metal tolerant bacteria isolated in this study was identified as *Klebsiella pneumoniae* (strain TAN-8) based on phylogenetic analysis of 16S rRNA sequence. The lag phase of *Klebsiella pneumoniae* was longer in arsenic stress condition than control while log and exponential phase were similar in both conditions. The bacterial strain was arsenic metabolizing bacteria and had no negative effects on root and shoot lengths of rice. The result of this experiment strongly supports the potential capability in *Klebsiella pneumoniae* strain TAN-8 of arsenic tolerance even at high concentration. The strain TAN-8 was capable of effective metabolism of arsenic and survives in high arsenic condition along with high temperature and pH. Thus, strain TAN-8 could be used for arsenic mitigation by reducing arsenic uptake by crops grown in Arsenic contaminated soil and could be used for other biotechnological applications.

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