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Original Research Paper

Identification and Characterization of the Effectively Arsenic Tolerant Bacterial Strains from the Potential Arsenic Contaminated site in 24-Parganas (North) District of West Bengal, India

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ABSTRACT

Arsenic is a common menace in the state of West Bengal particularly in the district of 24-Parganas (North) and (South). The major source of arsenic being geologic in nature. Soil samples collected from one such particular location Baduria were tested for the presence of arsenic (As), and on an average the As content of the soil was 2.18mg/kg. Different bacteria were isolated by serial dilution and were also tested for As tolerance. On the basis of their tolerance, three organisms (viz. C-7, A-2, A-1) have shown remarkable percentage uptake of As 43%, 22.17% and 14% respectively, which was highest for C-7 strain. Azithromycin and Streptomycin were found to be more effective to control C-7 with an inhibition zone of 4.5 cm, but for A-2 and A-1 it was Azithromycin with inhibition zone of 3.8cm and 3.5cm respectively. These organisms were characterized and identified by 16S rDNA technique, BLAST results and the cluster analysis by NTSYSpc ver. 2.02e program, and found to be *Bacillus* sp. strain CCBAU 51490 (C-7) (GenBank Accession Number EF377314.1), *Bacillus subtilis* sub sp. *subtilis* strain DSM 10 (Genbank Accession Number NR_027552.1) (A-2) and *Pseudomonas otidis* strain MCC 10330 (Genbank Accession Number NR_043289.1) (A-1). They can be used in the treatment of As contaminated sludge generated industrially from As treatment plant. This can give a new insight in microbial arsenic trapping with minimum toxic implications in the nature.

INTRODUCTION

Arsenic is a natural metalloid that is comparatively rare, but widely distributed in soils and rocks and also in the different water bodies. Numerous environmental factors influence arsenic speciation in soil, which include pH, redox potential, the presence of other ions, organic matter content, soil texture and fungal or bacterial activities (Turpinen et al. 1999). The sources of environmental arsenic may derive from various natural sources (i.e., weathered, volcanic, marine, sedimentary rocks, fossil fuels, minerals), water, air, living organisms and anthropogenic activities including mining, agricultural chemicals (e.g., pesticides, herbicides), wood preservatives, medicinal products, industrial activities (e.g., timber, tannery, paints, electroplating, etc.) (Dowdle et al. 1996, Liest et al. 2000, Mandal & Suzuki 2002). The most common arsenic species observed in the environment are the trivalent form arsenite [As(III)] and pentavalent form arsenate [As(V)] and it can occur in nature in its pure form as a black-grey metallic substance, but usually exists combined with other chemical elements (for example oxygen and sulphur) in a range of chemical compounds. Arsenic has been referred to as "essential toxins" because it is required in trace amounts for growth and metabolism though it is only toxic at high concentration, (Stolz et al. 2002). The toxicity of different forms of arsenic decrease in order: arsine>inorganic arsenite>organic arsenite>inorganic arsenate>organic arsenate>free arsenic (Mandal & Suzuki 2002) and it depends on factors such as physical state, gas, solution, or powder particle size, cell adsorption rate, elimination rate and nature of the chemical toxic compound (Anderson & Cook 2004).

The objectives of the present investigation include to determine the arsenic contamination in the soil; to isolate and characterize bacteria tolerant to arsenic; to find out the level of arsenic uptake and its binding site in the microbe; and to identify the bacteria by 16S rDNA technique.

MATERIALS AND METHODS

Arsenic analysis in soil: Soil samples were collected from the district of 24 Parganas (North) in the State of West Bengal. The soil samples were collected by the sterile spatula from the subsurface soil (from 0-15 cm in depth), placed in plastic bag and kept on ice or at 4ºC until further analysis.

Fifty gramme of the soil sample was added to 10 mL of 50% HNO_3. Then it was heated on a hot plate and filtered, and volume was made up to 10mL.

One to 2 g of soil samples were added to 10 mL 50% HNO_3, and heated and refluxed at 95°C for 10-15 min. The soil suspension was left at room temperature to cool, then

added with 5 mL concentrated HNO_3 and refluxed for another 30 min at 95°C. This step was repeated one more time. The solution was evaporated until approximately 5 mL left. After the solution cooled to room temperature, 2mL deionized H_2O and 3 mL 35% H_2O_2 were added and heated to 95°C. The solution was cooled to room temperature and added with 7 mL warm 35% H_2O_2. The solution was again cooled to room temperature, mixed with 5 mL concentrated HCl and 10 mL deionized H_2O and refluxed at 95°C for 15 min. The digested solution was filtered to remove residual particulates and then diluted to 100 mL with deionized H_2O. This acidified sample solution was used to measure the arsenic concentration by ICP-OES. It was nebulized and the resultant fine mist was carried out by an argon flow into a high temperature plasma generated by a radio frequency electric current running in an induction coil. The sample was decomposed in plasma, followed by atomized and excited and quantification of each compound was performed by monitoring a specific spectral line emitted by an atom (or ion). Continuous determination of arsenic was possible by monitoring the plasma with a polychrometer (Thermo Scientific, Model No. ICAP 6000 Duo). The most sensitive lines for arsenic lie in the UV region (193.759 nm) and the analysis was made by an appropriate spectrophotometer.

Bacterial isolation by serial dilution method: One gramme of soil sample was added to 10 mL of sterile water in the glass conical flask (Borosil). Then 1 mL of the suspension was added to another 10mL of sterile water in the test-tube (Borosil) and the process was repeated for another four times (i.e., up to 10^{-5} dilution). Pure culture was obtained by repeated sub-culturing technique from the isolated colonies from the 10^{-2} and 10^{-3} dilution plate.

Growth media for isolation of bacteria: The bacteria were grown in the nutrient medium containing 0.3g of beef extract, 0.5 g of peptone, 0.5g of NaCl and 2 g of agar in 100 mL of water. Pure culture was obtained by subculture and pure culturing technique from the isolated colonies. For each culture smears were prepared prior to Gram staining and cell morphology was investigated under the compound microscope (45X) (Dhar et al. 2004) and binding site of arsenic in the bacterial strains were detected by phase-contrast microscope (100X) (Leica, Model No. DM2500). Antibiotic sensitivity test was done by disk diffusion method and the bacterial strains were also characterized by catalase test.

Determination of minimum inhibitory concentration of arsenic by colorimetric assay method: Colorimetric assay method was used to determine the minimum tolerable concentration of the strains for both arsenate and arsenite (Dhar et al. 2004). The bacteria were grown in sodium arsenate containing nutrient broth. The media contained 10 mg/L, 20mg/L, 30mg/L, 50 mg/L, 70 mg/L, 80 mg/L and 100 mg/L concentrations of arsenic for the arsenic tolerant experiment of the bacterial strains. It was incubated at 37°C for 48 hrs. Optical density was measured at 580nm (Dhar et al. 2004).

Arsenic analysis of bacterial isolates: The bacterial cultures were centrifuged and the supernatants were taken for acid digestion. The supernatants were added with 10 mL 50% HNO_3, heated and refluxed at 95°C for 10-15 min. The suspension was left at room temperature to cool, then added with 5 mL concentrated HNO_3 and refluxed for another 30 min at 95°C. This step was repeated one more time. The analysis of arsenic was made by ICP-OES method just like to that for soil described earlier.

16S rDNA Sequence Analysis

1. DNA was isolated from the slant culture provided. Its quality was evaluated on 1.2% Agarose Gel, a single band of high molecular weight DNA was observed.
2. Fragment of 16S rDNA gene was amplified by PCR from the above isolated DNA. A single discrete PCR amplicon band of 1500 bp was observed when resolved on Agarose Gel.
3. PCR amplicon was purified to remove contaminants.
4. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 8F and 1492R primers using BDT v3.1 cycle sequencing kit on ABI 3730xl genetic analyser.
5. Reverse sequence of 546 bp of rDNA gene was used for further analysis.
6. The 16S rDNA gene was used to carry out BLAST with the nrdatabase of NCBI genbank database. The cluster analysis was done by UPGMA method through NTSYS pc ver.2.02e program.

RESULTS

Arsenic in soil samples: A total of 2 soil samples were collected from Baduria and Deganga. Arsenic concentration of the soil of Baduria and Deganga is 2.18mg/kg and 1.52mg/kg respectively.

Isolated bacteria: The bacterial strains were characterized from the pure cultures obtained by repeated sub-culturing technique from the isolated colonies (Table 1and Figs. 1a., 1b, 1c, 1d, 1e,1f). In 10^{-2} and 10^{-3} dilution plate the values of CFUs/mL were 37×10^3 and 32×10^3 respectively. Then the bacterial strains were characterized (Table 1 and Figs. 1a., 1b, 1c, 1d, 1e,1f).

Minimum inhibitory concentration of arsenic: Indigenous bacteria from arsenic contaminated soils were firstly enriched in the nutrient broth with increased arsenic concentrations from 10 mg/L to100mg/L. The LD_{50} doses of arsenic for C-7, A-1 and A-2 bacteria were found to be 50 mg/L,

Table 1: Characterization of bacteria.

Sample No.	Gram character of the bacteria	Dimension of the bacteria (μm)	Characterization of the bacteria by Catalase test	Antibiotic sensitivity test (Diameter of inhibition zone in cm)
C-7	Gram Positive long Rods	Length=5.69 Breadth=2	Catalase Positive	Sensitive to *Streptomycin* (4.5cm), *Azithromycin* (4.5cm)
A-1	Gram Negative short semi-rods	Diameter=1	Catalase Negative	Sensitive to *Streptomycin* (3cm), *Azithromycin* (3.5cm), Ciprofloxacin (3.1cm)
A-2	Gram-Positive long Rods	Length=3.14 Breadth=1	Catalase Positive	Sensitive to *Streptomycin* (3cm), *Azithromycin* (3.8cm), *Ciprofloxacin* (3.5cm), *Clindamycin* (3cm)

Table 2: Percentage uptake of arsenic by the bacteria.

Sample No.	Concentration of arsenic (mg/L)	Residual arsenic in the medium (mg/L)	Uptake of arsenic by the bacteria (mg/L)	% uptake of arsenic by the bacteria
C-7	50	28.5 ± 4.41	21.50	43.00
A-2	30	23.35 ± 3.61	6.65	22.17
A-1	10	8.56 ± 1.32	1.40	14.00

Table 3: BLAST result of C-7 bacteria.

Accession	Description	Max score	Total Score	Query coverage	E value	Max. ident
EF377314.1	*Bacillus* sp. CCBAU 51490 16S ribosomal RNA gene, partial sequence	2398	2398	96%	0.0	98%
HQ108182.1	*Bacillus cereus* strain BCM2 16S ribosomal RNA gene, partial sequence	2394	2394	96%	0.0	98%
GU384894.1	*Bacillus thuringiensis* strain ZJOU-010 16S ribosomal RNA gene, partial sequence	2394	2394	97%	0.0	98%
FJ686830.1	*Bacillus thuringiensis* strain NB8 16S ribosomal RNA gene, partial sequence					

Table 4: BLAST result of A-2 bacteria.

Accession	Description	Max score	Total Score	Query coverage	E value	Max. ident
NR_027552.1	*Bacillus subtilis* sub sp. *subtilis* strain DSM 10 16S ribosomal RNA partial sequence	2423	2423	96%	0.0	98%
NR_042638.1	*Brevibacterium halotolerans* strain DSM 8802 16S ribosomal RNA complete sequence	2412	2412	96%	0.0	97%
NR_024693.1	*Bacillus mojavensis* strain IFO15718 16S ribosomal RNA partial sequence	2412	2412	96%	0.0	97%
NR_024696.1	*Bacillus vallismortis* strain DSM11031 16S ribosomal RNA partial sequence	2396	2396	96%	0.0	97%

Table 5: BLAST result of A-1 bacteria.

Accession	Description	Max score	Total Score	Query coverage	E value	Max. ident
NR_043289.1	*Pseudomonas otitidis* strain MCC 10330 16S ribosomal RNA partial sequence	2143	2143	89%	0.0	96%
NR_025972.1	*Pseudomonas balearica* strain SP1402 16S ribosomal RNA partial sequence	2132	2132	89%	0.0	96%
NR_043419.1	*Pseudomonas alcaligenes* strain IAM12411 16S ribosomal RNA complete sequence	2109	2109	87%	0.0	97%
NR_026534.1	*Pseudomonas resinovorans* strain LMG2274 16S ribosomal RNA partial sequence	2106	2106	89%	0.0	95%

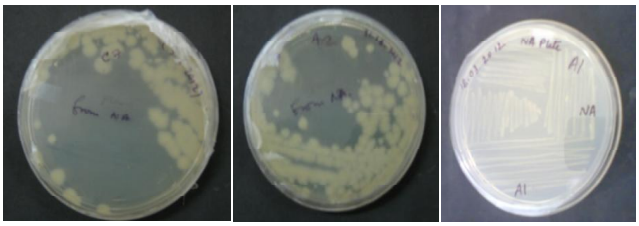

Fig. 1(a) Fig. 1(b) Fig. 1(c)

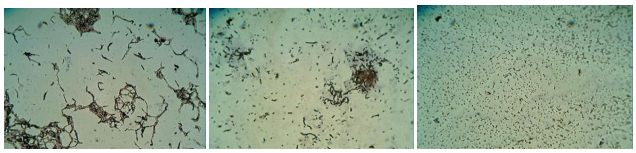

Fig. 1(d) Fig. 1(e) Fig. 1(f)

Fig.1: (a), (b) and (c). Bacterial isolates (C-7), (A-2) and (A-1) were grown on the nutrient agar media respectively. (d), (e) and (f) are photomicrograph of Gram+ve stained cells of arsenic resistant bacteria (C-7), (A-2) and Gram–ve stained cells of arsenic resistant bacteria (A-1) respectively under 45X magniffication of compound microscope.

10mg/L and 30mg/L of arsenic respectively (Fig. 3a, 3b, 3c).

Uptake of arsenic by the bacterial strains: C-7, A-2 and A-1 bacteria can tolerate up to 50, 30 and 10mg/L of arsenic respectively. The percentage uptake of arsenic of C-7, A-2 and A-1 bacteria is 43%, 22.17% and 14% respectively within 48 hours of incubation (Table 2), and the location of the binding site of the arsenic is the cytosol of the all bacterial strains and it was clearly observed by phase-contrast microscopy of C-7 bacteria (Fig. 2a, 2b).

Identification of arsenic absorbing isolates: Morphological and biochemical characteristics of all the three bacterial isolates was determined by Gram staining and biochemical tests. The investigation results indicated that two isolates were Gram positive rod shaped bacteria (C-7 and A-2) and the other one isolate (A-1) was Gram negative semi-rod shaped bacteria. The cultures which were labelled as C-7, A-2 and A-1 (closely related to) were found to be *Bacillus* sp. strain CCBAU 51490 (C-7) (GenBank Accession Number EF377 314.1), *Bacillus subtilis* subsp. *subtilis* strain DSM 10 (Genbank Accession Number NR_027552.1) and *Pseudomonas otidis* strain MCC 10330 (Genbank Accession Number NR_043289.1) respectively based on nucleotide homology and phylogenetic study by UPGMA cluster analysis by NTSYSpc ver.2.02e Program (Tables 3, 4, 5 and Figs. 4, 5, 6, 7, 8, 9).

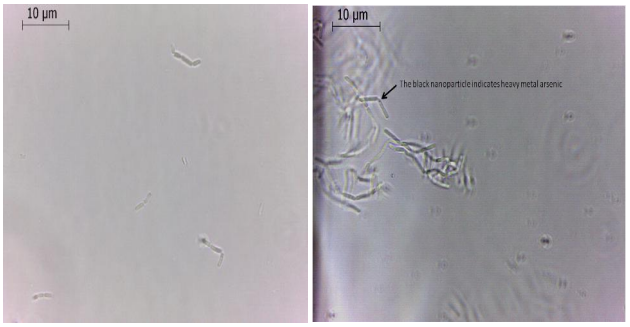

Fig. 2(a). Fig. 2(b).

Fig. 2(a) and (b): Photomicrograph of C-7 contol and treated C-7 Bacteria under 100X magnification of Phase-contrast microscope.

DISCUSSION

Arsenic in nature is specifically converted to different forms which will modify its toxicity level (Silver & Phung 2005). These chemical reactions are continuous in nature through precipitation or redox reaction (Gadd 1990, Lovely & Coates 1997) as well as protein-DNA adduct formation (Zhitkovitch & Costa 1992) and induction of stress proteins (Ballatori 1994). Other workers like Aksornchu et al. (2008), Shivaji et al. (2005) and Banerjee & Santra (2010) described the isolation of arsenic resistant bacterial strains from the soil of arsenic contaminated sites, their characteristics, arsenic tolerance ability i.e., the bacterial strains survived up to 40mM of sodium arsenite and 20mM and 100ppm of sodium arsenate containing culture media respectively. Aksornchu et al. (2008), Shakoori et al. (2010) and Shivaji et al. (2005) have identified the different arsenic tolerant bacteria by 16S rDNA sequence analysis and some of them are *Microbacterium oxidans*, *Achromobacter* sp., *Pseudomonas*, *Salmonella* spp., *Klebsiella oxytoca*, *Bacillus anthracis* and *Bacillus arsenicus* Con a/3^T (AJ606700).

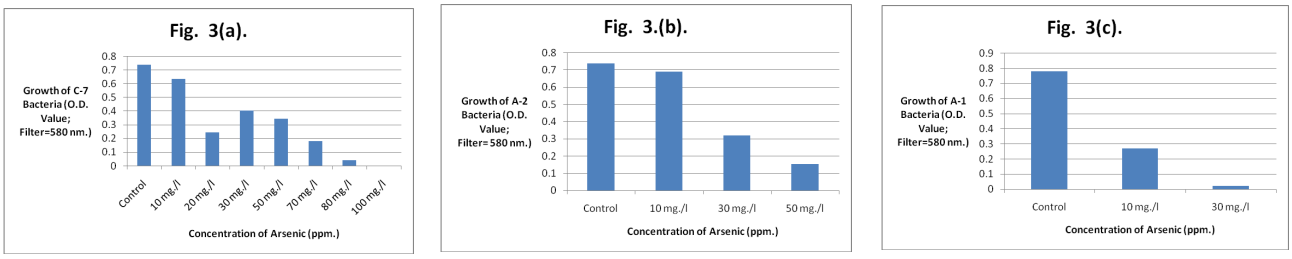

Fig. 3: Growth of arsenic tolerant bacteria (a) C-7, (b) A-2, (c) A-1.

CNNAAANNAACATGTTGGCTACTTATTGAAGTTAGCGGCGGACGGCGTTGAGTAACACGTGGGT
AACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAAC
CGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAG
CTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGC
CACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAAT
GGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGT
TGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCC
ACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTG
GGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAG
GGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGA
AATGCGTAGAGATATGGAGGAACACCAGTGGCNAAGGCGACTTTCTGGTCTGTAACTGACGCTG
AGGGCGCGAAAACGTGGGGAGCANACACGATTATATACCCTGGTANTCCACGTCGTAAACTATG
AGTGCCAAATGTTAGAGGGNTTTCCCCCCTCTAGTGCCGAAGTTAACGCATTAAGCACTCCGCC
TGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGCCCGCACAAGCGGTGGAG
CATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCT
AGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGT
CGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGT
TGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCA
TGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCG
AGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGA
AGCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACAC
ACCGCCCGTCACACCACGAGAGTTTGTAACATAGCGATGTCTCGTGGGGTCCCACTATCNNTCG
TNAA

Fig. 4: Consensus sequence of C-7 bacteria.

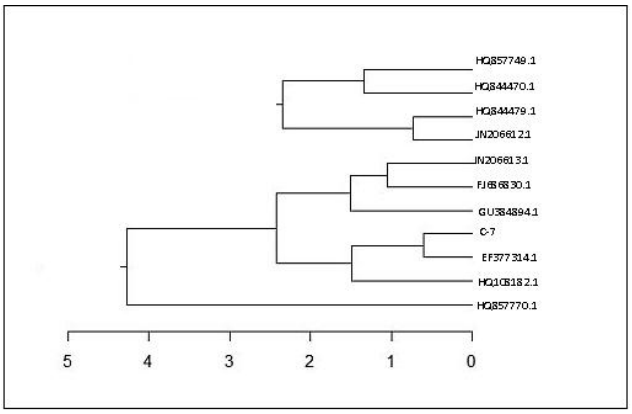

Fig. 5: Dendrogram of C-7 bacteria by UPGMA cluster analysis through NTSYSpc ver.2.02e Program.

GCTATAGGTGCTCTTGGAGCGCGCGGATTGGAGCTGGGTCCCTGAAGTTTGTGGCGGATG
GGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCT
AATACCGGAGGTTGTTTGAACCGCAGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTT
ACAGATGGACCCGCGGCGCATAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATG
CGTAGCCAACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTA
CGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCG
TGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTC
GAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCA
GCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCA
GGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAAC
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ACAATCCTAGAGATAGGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTT
GTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATTTTA
GTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACGCCGGTGACAAACCGGAGGAAGGTGG
GGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACCTGCTACAATGGACA
GAACAAAGGGCAGCGAAACCGCGAGGTTAAGCCAATCCCACAAATCTGTTCTCAGTTCGG
ATCGCCGTCTGCAACTCGACTGCGTGAAGCAGGAATCGCTTGTAATCGCGGATCAGCATG
CCGCGGTGAATACGTTCCCGGGCCTTGTACACACCCCCCGTCACACCACGAGAGTTTGTA
ACACCGGATGTCGGTGAGGTTACCTATCTGGAGCCGCCTACAGTGTAGAGGTCTACTTTG
CCC

Fig. 6: Consensus sequence of A-2 bacteria.

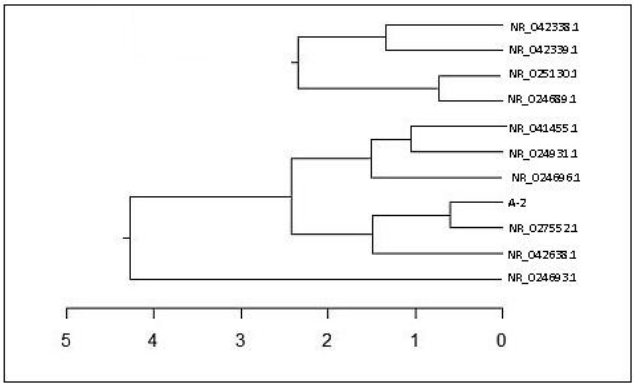

Fig. 7: Dendrogram of A-2 bacteria by UPGMA cluster analysis

GAGGTAGTATCGTATAGGTGAGCTTGGCTCTGACTGGTATATGGGGACCCCAGAGAGTGT
AGTTTTGGCTCGTGTGTATTAAATCGGCCGGGTAGTGGGGGATAACTCGGGGAAACTCGA
GCTAATACCGCAGACGTCCTACGGGAGAAAGCGGGGGATCTTCGGACCTCGCGCTATCAG
ATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAATGGCTCACCAAGGCGACGATCCGT
AACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGG
GAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGT
GTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAAT
ACCTTGCTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCG
CGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTG
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GAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATAT
AGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAA
AGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACT
AGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGT
ACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGT
TTAATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGG
ATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCGTCGTCAGCTCGTGTCGTGAGAT
GTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTGATGGTG
GGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCAT
CATGGCCCTTACGGCCAGGGCTACACACTGCTACAATGGTCGGTACAAAGGGTTGCCAAG
CCGCGAGGTGGAGCTAATCCCATAAAACCGATTGTAGTCCGGATCGCAGTTTGCAACTAG
AATGCGTGAAGTTGGAATCGCTCGTTATCCTGAATCAGAATGACACGGTGAATACGTTCC
CGGGCCTTGTACACACCCCCCAACACACCAAGGAGTGAGTGTTTACAGTTTCCCTGTCTT
TATAACTCGGCACCATGTACACTGTAGAGGTTCCTCTCCC

Fig. 8: Consensus sequence of A-1 bacteria.

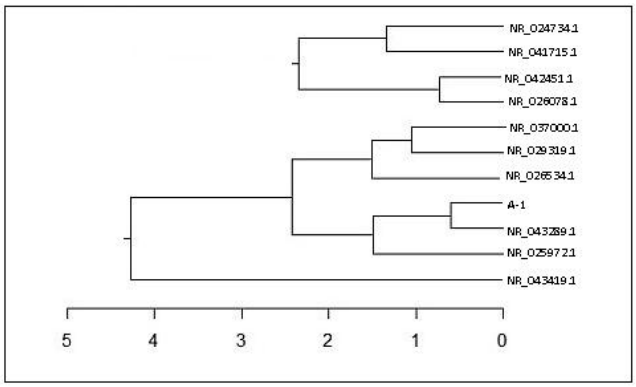

Fig. 9: Dendrogram of A-1 bacteria by UPGMA cluster analysis through NTSYSpc ver.2.02e program.

In the present study the tree bacteria (C-7, A-2, A-1) showed 43%, 22.17% and 14% uptake of As at 50mg/L, 30mg/L and 10mg/L concentration respectively and they were identified to be *Bacillus* sp. strain CCBAU, *Bacillus subtilis* subsp. *subtilis* strain DSM 10 and *Pseudomonas otidis* strain MCC 10330. On the basis of dendrogram analysis they belong to *Bacillus* and *Pseudomonas* cluster of organisms.

Antibiotic tolerance was reported by Berg et al. (2005). Similarly these bacteria were significantly resistant to ampicillin (C-7, A-1 and A-2), tetracycline (A-2), ciprofloxacin (C-7) and clindamycin (C-7 and A-1) and sensitive to streptomycin (diameter of inhibition zone of C-7, A-1 and A-2 were 4.5cm, 3cm and 3cm respectively), azithromycin (diameter of inhibition zone of C-7, A-1 and A-2 were 4.5cm, 3.5cm and 3.8cm respectively), ciprofloxacin (diameter of inhibition zone of A-1 and A-2 were 3.1cm and 3.5cm respectively) and clindamycin (diameter of inhibition zone of A-2 was 3cm).

These potential microbes can effectively be used in designing the effective bioremedial tower for purification of industrial sludge.

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