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RESEARCH ARTICLE

Efficacy of indigenous soil microbes in arsenic mitigation from contaminated alluvial soil of India

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Abstract Selected arsenic-volatilizing indigenous soil bacteria were isolated and their ability to form volatile arsenicals from toxic inorganic arsenic was assessed. Approximately 37 % of AsIII (under aerobic conditions) and 30 % AsV (under anaerobic conditions) were volatilized by new bacterial isolates in 3 days. In contrast to genetically modified organism, indigenous soil bacteria was capable of removing 16 % of arsenic from contaminated soil during 60 days incubation period while applied with a low-cost organic nutrient supplement (farm yard manure).

Keywords Soil bacteria · Arsenic · Volatilization · Bioremediation

Introduction

Arsenic (As) is an environmental threat of geogenic and anthropogenic origin. It is prevalent in many regions of the world (Tripathi et al. 2007; Mandal and Suzuki 2002), especially in the lower Ganges river Plain of Bengal, India (Rahman et al. 2005) and Bangladesh (Smith et al. 2000). Use of As-contaminated groundwater for irrigation made the soil a major sink, affecting farmland ecosystems (Williams et al. 2009).

The solubility, mobility, and bioavailability (and hence toxicity) of arsenic in soil systems depends on the chemical form, primarily the oxidation state. Estimation of total As fails to determine the species-dependent toxicity problem of the metalloid As (Heikens et al. 2007). Trivalent arsenic inactivates enzymes and affects the respiratory system, while pentavalent arsenic is rapidly excreted from the body. Generally, inorganic arsenic species are believed to be more toxic than organic forms to living organisms, including humans and other animals (Goessler and Kuehnett 2002; Meharg and Hartley-Whitaker 2002).The toxicity of different arsenic species were often reported to vary in an order of arsenite > arsenate > mono-methylarsonate (MMA) > dimethylarsinate (DMA) (Penrose 1974; Stugeron et al. 1989). For many years it was believed that the acute toxicity of inorganic arsenic was greater than organic arsenic and hence, the methylation of inorganic arsenic was a detoxification reaction. This dogma was held because DMA^{V}, the primary excreted metabolite of inorganic arsenic, is less acutely toxic than inorganic arsenic, until it was reported recently that derivatives of MMA^{III} (LD_{50} for mice is 2 mg $Askg^{-1}$ oral feed) are much more toxic than arsenite (LD_{50} for mice is 26 mg$Askg^{-1}$ of oral feed) (Hughes 2002).

Problems related to arsenic contamination have drawn attention worldwide and several physical and chemical remediation processes have been developed (Hering et al. 1996). However, these are expensive and have limited use as poverty and contamination coexist in most As-contaminated areas in the world (Zhu et al. 2009). Considering the limitations of conventional remediation techniques, biological methods using microbes, could be explored as alternative mitigation options (Singh et al. 2008; Chandraprabha et al. 2011).

Bioremediation of As by microorganisms has been widely hailed because of their potential advantage in providing a cost-effective, eco-friendly technology for heavy-metal removal (Valls and De Lorenzo 2002). Conversion of metal(loid)s to their volatile derivatives by organisms is a well-known phenomenon in nature (Challenger et al. 1945). During arsenic volatilization, some species of fungi and bacteria methylate inorganic As species to relatively less toxic volatile methylarsenicals (Rodriguez 1999; Cernansky et al. 2009).

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Arsenic biovolatilization starts with a reduction of AsV to AsIII and through a series of methylation reaction forms less toxic volatile organo-arsenicals (Turpeinen et al. 1999; Bentley and Chasteen 2002).

Formation of gaseous As has been reported (Abedin et al. 2002; Mahimairaja et al. 2005). As summarized by WHO (WHO 2001a, b), volatilization can substantially contribute to As removal from top soils, up to 12–35 % $year^{-1}$. Several species of soil dwelling microorganisms have shown As-volatizing potential (Frankenberger 2002; Turpeinen et al. 2002, Islam et al. 2007; Mohapatra et al. 2008). This includes species of *Penicillium* and *Aspergillus*, which can volatilize both organic and inorganic As compounds, while different species of *Pseudomonas* were capable of volatilizing inorganic As. The rate of biovolatilization of AsV was observed to be 23 % while for AsIII it was 26 % by *Staphylococcus* sp. in 3 days from culture media (Srivastava et al. 2012). Most of the previous reports related to As volatilization involved either aerobic (*Pseudomonas*, *Bacillus*, and *Alcaligenes*) or anaerobic (*Clostridium*, *Desulfovibrio*, and *Methanobacterium*) bacteria (Bentley and Chasteen 2002).

Earlier attempts made to assess the capabilities of microorganism in decontaminating soil matrices have often dealt with genetically engineered organisms (Liu et al. 2011) or in simulated in situ situations of anthropogenic contamination. The As contamination in West Bengal is characteristically different, geogenic in origin, and widespread across waterlogged rice ecosystems which poses an unique soil micro-environment of staggered anaerobic and aerobic spells. No specific data for anaerobic soils such as flooded paddy fields have been reported, making it yet not possible to assess the importance of As behavior in paddy fields (Abedin et al. 2002; Mahimairaja et al. 2005). Thus, the present investigation has been aimed to characterize the arsenic decontaminating microorganisms, naturally isolated from the soils of the endemic area. The mechanism which coincidentally appears to regulate As may have evolved in an arsenic-rich environment and may help stake-holders of the contaminated area, principally marginal in socio-economic stratum, to provide for a low-cost, user-friendly technology for arsenic mitigation. To reach such an outcome the present study has been designed with the specific objectives to identify efficient As-volatilizing soil microbes isolated from the polluted soil and to evaluate their volatilizing potential when enriched with farm yard manure and in contaminated aerobic and anaerobic soil systems.

Materials and methods

Isolation and screening of arsenic-volatilizing microbes

Arsenic-contaminated surface soils (0–15 cm) were collected from different sites of Chakdah block of Nadia district, India. Total and extractable As concentrations were estimated (Sparks et al. 2006). For isolation and purification of As-resistant bacterial strains, diluted soil samples prepared in sterile saline solution, were plated on solidified Luria Bertani (LB) medium (an enriched medium for growth of the isolates under stressed conditions) with either 500 mg L^{-1} AsIII or 1,000 mg L^{-1} AsV and incubated at 30 °C for 48 h. Arsenic volatilization capacities of 65 purified bacterial isolates were analyzed by a modified trapping method (Gao and Burau 1997). Single colonies of As-resistant bacteria were grown in closed vials containing 50 mL LB medium with 25 mg L^{-1} AsV incubated at 30 °C for 3 days and capped with mercuric nitrate impregnated filter paper (Edvantoro et al. 2004). Trapping material (filter paper) was prepared by soaking Whatman 541 cellulose filter paper with $Hg_2(NO_3)_2 \cdot 2H_2O$ in 10 % acetic acid water for 3 h and then air-dried (Pearce et al. 1998). Volatilized As was trapped in filter paper and analyzed for total As in an atomic absorption spectrophotometer (PerkinElmer, USA) coupled with FIAS 400 (Sarkar et al. 2012).

Tolerance to inorganic arsenic

Tolerance to AsV and AsIII were determined for each isolate by growing them separately in 20 mL LB liquid medium spiked with increasing concentrations of AsV (0 to 500 mM) and AsIII (0 to 100 mM) and incubated for 48 h at 30 °C. Controls for each concentration were inoculated with cell suspension grown in LB medium without As to obtain a cell density of approximately 10^6 CFU (colony forming unit) mL^{-1}. Bacterial growth was monitored by measuring the optical density at 600 nm with a UV–vis spectrophotometer (Varian Cary-50 UV–vis). Bacterial isolates that could tolerate the highest As concentrations were selected and identified by their morphological features and biochemical properties (Holtz 1993).

Identification of the bacterial isolates based on 16S rDNA sequence analysis

Bacterial isolates showing considerable AsIII-volatilizing ability and higher As tolerance capacity were selected for molecular identification by 16S rDNA sequence analysis. Total genomic DNA was extracted (Sambrook 2001), and PCR amplification of 16S rRNA gene with forward primer 27F 5′-AGA GTT TGA TCM TGG CTC AG-3′ and the reverse primer 1492R 5′-GGY TAC CTT GTT ACG ACT-3′ (Chroumous Biotech Private Limited, India) were carried out. The reaction mixture composed of 1X PCR buffer, 0.2 mM dNTPs, 10 pmole of each primer, 60 ng of DNA template, 2 units of Taq DNA polymerase, and sterile deionized water to a final volume of 25 μL. Before amplification cycle, DNA was initially denatured at 94 °C for 5 min followed by 1 min

denaturation at 94 °C. After amplification, a final extension step (10 min at 72 °C) was performed. The cycling parameters consisted of 35 cycles initiated through denaturation at 94 °C for 5 min, denaturation at 94 °C for 1 min, primer annealing at 52 °C for 1 min, and extension at 72 °C for 5 min. The PCR products were purified and held at 4 °C until verification through agarose gel electrophoresis (1 %).

The amplified and gel-eluted PCR fragments of the rDNA were sequenced in ABI 3100 Genetic Analyzer with primers 27F. Sequencing reaction was performed by using the Big Dye terminator cycle sequencing Kit V3.1 (Applied Biosystems, Foster City, USA) following the manufacturer's protocol. The partial 16S rDNA sequences of the isolated strains were compared with those available in the GenBank database by BLASTN algorithm to identify sequences with a high degree (≥98 %) of similarity.

Biovolatilization of arsenic by efficient bacteria in culture medium

Volatilization of inorganic As species mediated through selected bacterial strains were estimated in aerobic and anaerobic situations. Volatilized As species were trapped in mercurous-nitrate-impregnated filter paper (Gao and Burau 1997). To investigate As volatilization, LB medium was spiked with AsV and AsIII (5, 10, 15, 20, 25, and 30 mgL^{-1}) and incubated with different bacterial strains in closed vials with mercurous-nitrate-soaked filter papers as As-trapping device. Sodium arsenate (Na_2HAsO_4, $7H_2O$) and sodium arsenite ($NaAsO_2$) were used. Freshly prepared 100 μL cells of each culture media ($10^6 CFU\,mL^{-1}$) were added in 25 mL LB medium in filter paper capped closed vessel, incubated at room temperature 25±3 °C for 3 days (Liu et al. 2011). Anaerobic condition was maintained through N_2 sparging for 2 min to displace oxygen and the bottles were prepared under N_2 atmosphere. Each set of experiments was established in triplicate and total As content of the trapped material was measured (Sarkar et al. 2012).

Arsenic species identification during volatilization

A microwave digestion system (Multiwave 3000, Anton Par) with a rotor of 48 Teflon digestion vessels was used for sample digestion and extraction. For total As analysis 5 mL of culture media and trapped filter paper disk were taken in a clean Teflon digestion vessel and 5 mL aqua regia was added to it. The vessel was then digested in a microwave followed by a 5-min hold and analyzed by inductively coupled plasma–mass spectrometry (ICP-MS; PerkinElmer ELAN DRCe 6000) for total As. For speciation analysis 5 mL of culture media (culture broth of the previous experiment spiked with 10 mgL^{-1} AsV was taken) and the trapped filter paper added with 10 mL of orthophosphoric acid (1 M) with ascorbic acid and placed in a microwave teflon vessel and the mixture was maintained at 60 W for 10 min and subjected for separation through an anion exchange column (Hamilton PRP-X100 F with a precolumn used for chromatographic separation). For the isocratic method, a PerkinElmer Series 200 Micro Pump was used instead of the quaternary pump and the detections and quantifications have been done through a PerkinElmer ELAN DRCe ICP-MS.

Volatilization of arsenic from arsenic-contaminated soil

Arsenic-contaminated soil used for this experimental study was collected form Ghentugachi (N 23°02′04.4″ and E 088° 34′55.5″) village of Nadia district, India. Total As concentration of the soil is 27 $mgkg^{-1}$ and extractable As concentration is 7 $mgkg^{-1}$ pH 7.51. To determine As volatilization by efficient bacterial strains, microcosm experiments were performed with 500 g of air-dried test soil in teflon-coated closed bottles through incubation with 5 mL culture of different bacterial strains. Formaldehyde (0.04 %) was used to kill the microbes in control soil. It inhibits the microbial activity but does not change the pH and dissolved nutrient concentration of the soil. Farm yard manure was used as organic source and added at 0, 2.5, and 5 gkg^{-1} and thoroughly mixed to 500 g of soil. Volatilized As evolved from the soils was trapped in mercurous-nitrate-impregnated filter papers placed in the caps of closed bottles, incubated at room temperature (25±3 °C) for 30 and 60 days. Each experiment was performed in triplicate and appropriate moisture conditions of the soils were maintained according to the method of Edvantero et al. (2004). Arsenic trapped filter papers were removed at 10-day intervals and total soil As concentration was determined through the standard method (Sparks et al. 2006) at the end of the incubation period.

Results

Arsenic tolerance and molecular identification of bacterial strains

Selected bacterial strains, isolated from contaminated soil showed considerable resistance to As toxicity with varying levels of tolerance capacity (Table 1). Twelve bacterial isolates, having As-volatilizing activity, were subjected to As resistance tests and most of them were found tolerant to As concentration higher than 100 mM. The minimal inhibitory concentration (MIC) of inorganic As for selected strains were observed within a range of 106.6–400 mM for AsV and 10–53.3 mM for AsIII while highest tolerance to AsV

Table 1 Identification and arsenic tolerance of arsenic-volatilizing soil microbes

Bacterial isolates	MIC of AsV (mM)	MIC of AsIII (mM)	GenBank accession no.	Most closely related organism based on 16S rRNA gene sequence
ADP-03	160.0	21.3	AB697184	*Rhodobacter sphaeroides*
ADP-33	166.7	13.3	AB697486	*Rhodobacter sphaeroides*
AMT-08	400.0	46.7	AB694007	*Rhodobacter sphaeroides*
ADP-18	246.7	40.0	AB697485	*Bordetella* sp
ADP-28	146.7	24.7	AB697181	*Alcaligenes faecalis* subsp. *faecalis strain*
ADP-32	320.0	20.0	AB697182	*Alcaligenes* sp.
AGH-04	193.3	16.7	AB697483	*Alcaligenes* sp
AGH-09	380.0	33.3	AB694008	*YCY20* (unclassified bacterium)
AGH-13	106.7	13.3	AB694010	*MPBA40* (unclassified bacterium)
AGO-S15	120.0	10.0	AB697183	*Alcaligenes* sp
AMT-03	160.0	21.3	AB696983	*Bacillus clausii*
AMT-04	213.3	53.3	AB694009	*Alcaligenes faecalis*

MIC minimal inhibitory concentration, *AsV* arsenate, *AsIII* arsenite

(400 mM) and AsIII (53.3 mM) was exhibited by the isolate AMT-08 and AMT-04, respectively. Molecular characterization of the strains has been carried out based on sequencing of 16S rDNA and compared with existing GenBank databases (Table 1).

Biovolatilization of arsenic by efficient bacteria in culture medium

Incubation studies with 12 selected As-volatilizing bacterial strains were conducted in cultures spiked with 25 mgL^{-1} AsV for 72 h under aerobic and anaerobic condition to assess their As-volatilizing efficiency (Fig. 1). The bacterial isolates AMT-08 and AGH-09 showed higher As-volatilizing capacity under aerobic conditions, ADP-18 volatilized maximum As under anaerobic conditions while AMT-04 performed well in both conditions.

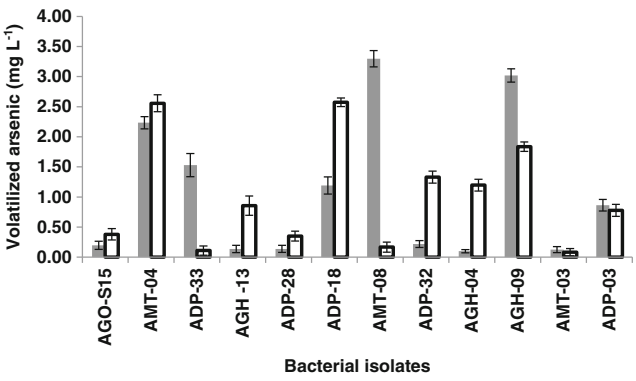

Fig 1 Volatilization of arsenic by isolated soil microbes in 3 days of incubation under aerobic (*shaded bars*) and anaerobic (*unshaded bars*) conditions (*error bar* represents the standard error, $N=3$)

The As volatilization capacity of the selected bacterial strains has been initiated across a range of inorganic AsIII and AsV spiked in the system (Table 2). AMT-08 showed highest efficiency of removing both AsIII and AsV from culture media spiked with 5 mgL^{-1} of AsIII/AsV in aerobic situation while such efficiencies tend to decrease with increasing concentration of As in media (Table 2). Similar efficiencies have been shown by ADP-18 under anaerobic situation which volatilized about 35 % AsIII and 30 % AsV. Interestingly, AMT-04 simultaneously supported considerable arsenic volatilization from aerobic as well as anaerobic systems. It is a characteristic feature of facultative aerobe, showing better performance under anaerobic environment but lesser activity under aerobic condition.

Arsenic species identification during volatilization under aerobic system

Arsenic speciation has been measured by high-performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (Hymer and Caruso 2004; Sanz et al. 2007).

Arsenic speciation studies in culture broth (cells + LB medium; Table 3) and the filter trap (Table 4) showed recoveries up to 87 % of total As. Highest percent recovery in trapping filter paper was observed with strain ADP-18. All the As species recovered were AsIII and AsV with DMA and MMA at non-detectable levels. Significant proportions of AsIII have been recovered from the culture broth when the media has been solely spiked by AsV.

It is interesting to note that the As trapped in the filter paper did not show any recoveries of AsIII or DMA, MMA, arsenobetaine (As-B) which envisaged that whatever As has been volatilized during incubation was in the form of AsV, although, at the same time, AsIII and AsV coexist in the

Table 2 Arsenite (AsIII) and arsenate (AsV) volatilization (mgL^{-1}) through bacterial cultures exposed to varying concentrations in 3 days of incubation

	Aerobic					Anaerobic			
	Uninoculated control	AGH-09	AMT-04	AMT-08	Percent volatalization	Uninoculated control	ADP-18	AMT-04	Percent volatalization
AsIII (mgL^{-1})									
5	BDL	1.72±0.10	1.42±0.09	1.86±0.12	25.00 a	BDL	1.76±0.08	1.5±0.06	21.73 a
10	0.2±0.02	2.82±0.13	1.88±0.10	3.01±0.12	19.78 ab	0.25±0.03	2.98±0.03	2.65±0.09	19.60 a
15	0.32±0.02	3.2±0.10	2.01±0.11	3.56±0.26	12.78 bc	0.42±0.01	4.30±0.26	3.5±0.15	18.26 ab
20	0.41±0.02	3.74±0.10	2.4±0.11	4.37±0.16	13.65 bc	0.51±0.02	5.33±0.08	3.61±0.15	15.75 ab
25	0.52±0.02	3.98±0.09	3.73±0.17	4.92±0.11	14.15 bc	0.64±0.04	3.60±0.18	3.73±0.10	10.63 ab
30	0.65±0.03	3.1±0.18	2.82±0.10	3.66±0.06	8.52 c	0.8±0.03	2.7±0.12	3.03±0.19	7.25 b
AsV (mgL^{-1})									
5	BDL	0.98±0.04	0.90±0.02	1.27±0.02	15.75 a	BDL	1.5±0.22	1.3±0.12	18.67 a
10	0.31±0.02	1.35±0.11	0.94±0.04	1.85±0.09	11.13 a	0.28±0.05	1.8±0.03	1.57±0.04	12.17 ab
15	0.43±0.03	1.85±0.11	1.11±0.07	2.65±0.15	10.06 a	0.36±0.03	1.98±0.05	1.85±0.04	9.31 b
20	0.56±0.03	2.73±0.18	1.88±0.06	3.4±0.05	10.71 a	0.47±0.01	2.6±0.03	1.91±0.02	8.30 b
25	0.77±0.07	3.5±0.31	2.44±0.06	3.8±0.12	10.51 a	0.61±0.02	3.85±0.04	2.97±0.17	9.91 b
30	0.81±0.05	2.5±0.28	1.21±0.05	2.74±0.03	4.37 b	0.88±0.03	3.12±0.11	2.73±0.11	7.48 b

Figures denoted by letters are statistically different at 5 % probability level. M±SD (N=3)

NB arsenic volatilizations were determined in tri-acid digested filter trap, *BDL* below detectable limits

Table 3 Arsenic species recoveries from cells and LB medium (media spiked with 10 mg L^{-1} AsV)

Bacterial isolates	AsIII (mg L^{-1})	AsV (mg L^{-1})	Species sum (mg L^{-1})	Total As (mg L^{-1})	Percent transformation (AsV to AsIII)	Recovery (%)
Uninoculated control	ND	6.12 a	6.12 ab	8.65 a	–	70.8 b
ADP-18	4.65 a	1.94 c	6.59 a	9.21 a	46.5 b	71.6 ab
AGH-09	3.34 b	4.13 b	7.47 a	9.40 a	33.4 c	79.5 a
AMT-04	1.42 c	2.89 bc	4.31 b	8.60 a	14.2 d	50.1 c
AMT-08	5.40 a	ND	5.40 ab	8.20 a	54.2 a	65.9 b

Figures denoted by letters are statistically different at 5 % probability level. M±SD (N=3)

ND not detected

culture broth at the end of incubation inoculated with almost all bacterial strains. The unaccounted part of total As which could not be retrieved by summing up the individual species, may be due to other As species (DMA, MMA, As-B etc.) in nondetectable proportions both in filter-trapped accumulation and in the cell + LB medium.

Arsenic removal from contaminated soil by microbial volatilization

To take an account of in situ As volatilization efficiencies of selected bacterial strains, contaminated soils were incubated for 30 and 60 days with efficient As-volatilizing bacterial inoculants with or without organic supplementation through farm yard manure. Significant variations in As volatilization were observed with varying inoculants and organic supplementation as compared to respective controls (Table 5). Arsenic removal from the soil system through volatilization by bacterial strains were observed to increase with advancement of incubation period irrespective of the bacterial strains involved which has been further stimulated with increasing application of organic amendment.

AMT-08 has been found most successful in managing removal of the metalloid from soil throughout the entire incubation period (Fig. 2) and recorded a maximum 4.22 mg kg^{-1} at 60th day of incubation when the soil has been amended with 5 g kg^{-1} FYM of soil. It is interesting to note that significant arsenic recoveries in filter traps were also obtained from un-inoculated controls when amended with FYM at varying levels.

Discussion

Arsenic tolerance

Microbes developed various intrinsic As tolerance mechanism to sustain in the adverse environmental conditions. Metal resistant of bacteria often have genes located on plasmids. Genetic system named *ars* operon is the main functional unit for As resistance (Mukhopadhyay and Rosen 2002). *ars*R is the repressor of the operon, *ars*B can pump out AsIII present within the cell, *ars*C is the AsV reductase (Joshi et al. 2009). The bacterial strains isolated and studied through the present investigation have shown much higher resistance to AsV than those isolated from soil, gold mines, and geothermal effluents in the related researches throughout world (Saltikov and Olson 2002; Simeonova et al. 2004). The bacterial strains under the present investigation were isolated from anaerobic soil environment (submerged paddy soil) which is predominated by AsIII over AsV and hence showed much higher tolerance to AsIII as compared to findings from related research established as a model microorganism for bioremediation of arsenic and one of the most arsenic-resistant microorganisms (400 mM for AsV and 60 mM for AsIII) described to date (Mateos et al. 2006). The selected tolerant genera, *Rhodobacter sphaeroides* and *Alcaligenes faecalis* have not been previously isolated from arsenic-contaminated soil environments and no report exists about their role in arsenic bioremediation. Bacteria capable of arsenic volatilization isolated herein are genetically diverse which is in conformity with previous findings (Bentley and Chasteen 2002).

Table 4 Arsenic species recoveries in filter paper trap

Bacterial isolates	AsIII (mg L^{-1})	AsV (mg L^{-1})	Species sum (mg L^{-1})	Total As (mg L^{-1})	Recovery (%)
Uninoculated control	ND	ND	ND	ND	–
ADP-18	ND	1.58 ab	1.58 a	1.80 ab	87.7 a
AGH-09	ND	2.06 a	2.06 a	2.81 a	73.3 c
AMT-04	ND	1.67 ab	1.67 a	2.54 a	65.7 d
AMT-08	ND	2.51 a	2.51 a	3.22 a	77.9 b

Figures denoted by letters are statistically different at 5 % probability level. M±SD (N=3)

ND not detected

Table 5 Arsenic volatilization (mgL^{-1}) from soil mediated through bacterial strains with organic supplementation

Bacterial isolates	Volatilized trapped As (mgL^{-1})					
	30 days			60 days		
	Farm yard manure (gkg^{-1} of soil) 0	Farm yard manure (gkg^{-1} of soil) 2.5	Farm yard manure (gkg^{-1} of soil) 5	Farm yard manure (gkg^{-1} of soil) 0	Farm yard manure (gkg^{-1} of soil) 2.5	Farm yard manure (gkg^{-1} of soil) 5.0
Uninoculated control	0.23 d	0.51 d	0.78 d	0.45 d	0.83 c	1.01 d
ADP-18	0.44 c	0.82 c	1.23 cd	0.76 c	1.01 c	1.64 cd
AGH-09	0.76 b	1.19 b	2.12 b	1.13 b	2.03 b	3.16 b
AMT-04	0.66 b	0.94 bc	1.67 bc	0.91 bc	1.78 b	2.37 bc
AMT-08	0.98 a	1.60 a	2.74 a	1.35 a	2.92 a	4.22 a

Figures denoted by letters are statistically different at 5 % probability level. M±SD (N=3)

NB arsenic volatilizations were determined in acid digested filter trap

Bacteria mediated arsenic volatilization from culture media

Biovolatilization of As from culture media inoculated with 12 selected bacterial strains showed (Fig. 1) varying capabilities of As volatilization exhibited by different strains under aerobic and anaerobic conditions. Some strains (AMT-04, ADP-03) remained versatile in both the systems (aerobic and anaerobic). Similar observations have also been recorded by Mohapatra et al. (2008) who found that As volatilization decreased with increase in concentration of AsV and reached equilibrium after certain period of time.

The bacterial volatilization of As also depends on substrate and AsIII volatilization rates have been found to be relatively higher than AsV both from aerobic and anaerobic systems (Table 2, Fig. 3). Some microbes can take up AsV via phosphate transporter and then reduce AsV internally to AsIII which is then extruded from the cell (Joshi et al. 2009). This may be the probable reason of getting AsIII though we started the experiment with sole AsV. In case of methylation AsIII is a reaction intermediate of AsV reduction to MMA, DMA, TMA, and supposed to be more readily reduced than AsV. The rate of biovolatilization was 23 % (AsV) and 26 % (AsIII) in case of *Staphylococcus* sp (Srivastava et al. 2012) also supported the present findings. Fungal strains, *Aspergillus niger*, *Aspergillus clavatus*, and *Neosartorya fischeri* on the contrary, did not show any mentionable difference in volatilization output from AsIII or AsV system (Cernansky et al. 2009). This investigation identified the bacterial strain having As volatilization potential in both aerobic and anaerobic conditions from different inorganic As species.

Bacteria mediated arsenic volatilization from soil

With a view to develop an efficient biological tool for decontaminating As in soil matrices, volatilization was checked in naturally contaminated soil. Selected bacterial

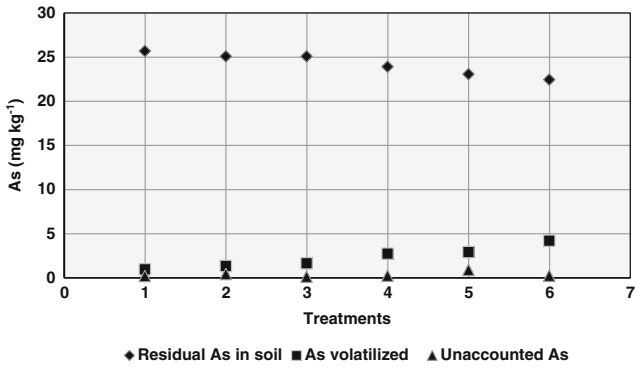

Fig. 2 Arsenic volatilization from soil inoculated with bacterial strain AMT-08 and amended with farm yard manure. The treatments were soil amendment through Farm Yard Manure at 0, 2.5, and 5 gkg^{-1} of soils at 30 days (*1*, *2*, and *3*) and at 60 days (*4*, *5*, and *6*)

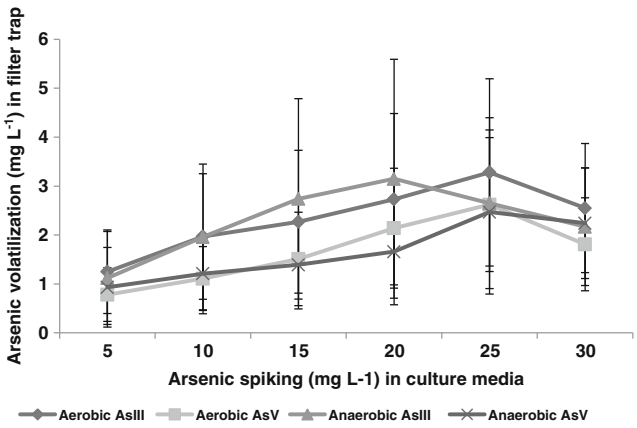

Fig. 3 Arsenic volatilization (mgL^{-1}) by As-volatilizing soil microbes exposed to varying concentration of AsV and AsIII in 3 days of incubation under aerobic and anaerobic systems (*error bar* represents the standard error, N=3)

strains used in the present investigation showed varying capabilities of removing As from soil systems through volatilization which has been further stimulated when supplemented by external application of organic. These observations were supported by previous research reports that supplemented C source, which provided additional energy for microbes, was able to stimulate As volatilization (Edvantoro et al. 2004; Lee et al. 2005). Unlike other incubation study experiments, where the authors had sterilized the soils prior to microcosm inoculation (Turpeinen et al. 2002), soils were not sterilized in this study. We had tried to assess volatilization of As from soils by microcosms consisting of As-volatilizing soil bacteria under natural condition in presence of native microbial residents of soil. This would imitate the true conditions when the microcosms would be applied for As mitigation in contaminated area, since it would depict the competitive existence and volatizing activity of the applied bacterial strains in presence of other native soil bacteria.

Wide range of microorganisms, belonging to *Methanobacterium*, *Bacillus*, *Pseudomonas*, *Streptococcus*, *Staphylococcus*, *Aspergillus*, *Penicillium*, and *Scopulariopsis* strains, have been identified as potential producers of volatile As (Cullen and Reimer 1989). In the present investigation an indigenous soil bacterial strain AMT-08 have the ability to remove 10 % (in 30 days) and 16 % (in 60 days) As when supplemented with farm yard manure. Similarly, Woolson (1977) reported 18 % loss of As in 160 days under aerobic conditions, from artificially As spiked soils, whereas the reported strain AMT-08 is capable of removing twice As per day from soil. The strain AMT-08 has an increased As-volatilizing ability with exogenous nutrients (farm yard manure) and successful exploitation of these hyper-tolerant isolates may deliver an eco-friendly tool for As mitigation within manageable expenditure as compared to genetically engineered alternatives.

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