Dynamic Biochemistry, Process Biotechnology and Molecular Biology ©2012 Global Science Books

Characterization of Efficient Arsenic-Removing Bacteria from *In-vitro* Conditions

Aparajita Majumder* • **Supradip Sarkar** • **Subhash Chandra Kole**

Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, 741235, India

Corresponding author: * **aparajita_micro@yahoo.co.in**

ABSTRACT

A total of 20 arsenic-tolerant bacterial strains were isolated from arsenic-contaminated soil of eastern India by using basal salt minimal medium (BSMYI) medium supplemented with 150-500 mg l^{-1} of arsenate (As^V). Among them, 10 isolates exhibited higher arsenic resistance and could grow in up to 12,000 mg l^{-1} of arsenate (As^V) and 200 mg l^{-1} of arsenite (As^{III}). Those 10 isolates were incubated for 3 days in BSMYI medium containing 25 mg l^{-1} of both As^V and As^{III} separately to assess their ability to remove arsenic. The isolates could remove 1.0-6.4 mg l^{-1} of As^V and 2.0-7.6 mg l^{-1} of As^{III} from the arsenic-amended media. The bacterial isolate AGH-21 showed the highest arsenic-removing capacity, both for As^V (25.6%) and As^{III} (30.4%). The isolated pure culture showed white irregular undulating colonies on a BSMYI agar slant and was an encapsulated endospore-forming Gram-positive bacterium. Molecular characterization of the strain based on sequencing of 16SrDNA and subsequent comparison with existing databases identified the organism as *Bacillus flexus*.

Keywords: arsenate, arsenite, *Bacillus flexus*, BSMYI media, removing ability
Abbreviations: **As^{III}**, arsenite; **As^V**, arsenate; **AGH**, arsenic ghentugachi; **BSMYI**, basal salt minimal media I; **PCR**, polymerase chain reaction

INTRODUCTION

Arsenic, one of the most prevalent toxic metalloids in the environment, has a long and nefarious history; its very name has become synonymous with poison. Despite its notoriety as a deadly poison, arsenic, at a lower concentration, helps to carry out different metabolical processes of different biological systems. But at a higher concentration the same becomes toxic, and is thus termed an 'essential toxin' (Krumova *et al.* 2008). Due to its ability to induce chromosomal aberration during DNA replication, arsenic is considered as a human carcinogen and a potential mutagenic agent. It also interferes with the DNA repair system, signal transduction pathways and inhibits many enzymatic activities and also damages the respiratory, digestive, circulatory and nervous systems (Rehman *et al.* 2010). Groundwater pollution by arsenic in different parts of West Bengal is a major public health problem. In the state of West Bengal, India, 111 endemic blocks located primarily in 12 districts adjoining to river Bhagirathi as well as the contiguous districts of Bangladesh along river Padma and its basin is of great concern (Sinha *et al.* 2011). Arsenic intoxication by humans through drinking has received most attention; however, little attention has been paid to arsenic toxicity in the water-soil-plant chain. Initially soil acts as a major sink, although continuous addition of arsenic through polluted irrigation water also makes the soil a source and transports it to the edible part of crops (Mukhopadhyay and Sanyal 2004). Besides drinking water, the intake of arsenic through the food chain has been established (Meharg *et al*. 2009). Consumption of such food material caused catastrophic health hazards among humans (Guhamazumder 2008). Microorganisms, in general, and bacteria, in particular, have developed certain mechanisms to avoid arsenic toxicity. Some of them also have the ability to transform more toxic (arsenite, As^{III}) into less toxic (arsenate, AS^V) forms. Bacterial methylation of arsenic has great environmental significance for biological removal of arsenic. Inorganic arsenic is biomethylated to both volatile species such as monomethylarsine (MMA), dimethylarsine (DMA), and trimethylarsine (TMA) and non-volatile species such as methylarsonic acid, dimethylarsinic acid, and trimethylarsenic oxide (TMAO). In recent years, arsenic biomethylation is considered as a potential way of detoxification of arsenic in the environment, as the toxicity of organic arsenic is much more than that of its inorganic form (Krumova *et al.* 2008). There has been increasing interest in the application of bioremediation to mitigate arsenic toxicity in soil systems. Some bacterial isolates can sustain higher arsenic concentrations (Rehman *et al*. 2010) and such isolates can be used to reduce the arsenic load of polluted soil. Based on this background, a controlled laboratory experiment was conducted to remove arsenic from arsenic-rich liquid media by different bacterial isolates that were isolated from arsenic-contaminated soils of Nadia district, India.

MATERIALS AND METHODS

Study site and soil properties

Baseline survey data reveals that total arsenic status of the surface (0-150 mm) soils of Ghentugachi village (N 23° 2′ 4″ and E 88° 34′ 55.5″), Nadia district, West Bengal, India ranging between 7.6 and 16.63 mg kg^{-1}. During July, 2009 soils were collected from 24 farmers' fields of a jute-growing village. The soil of the field was silty loam and arsenic concentration was measured (Sinha *et al.* 2011). The total arsenic status of the ground water was in the range of 0.146 to 0.573 with a mean value of 0.231, which is 23 times higher than the safe limit of arsenic toxicity as defined by the WHO (1996). The total arsenic content of the soil was between 7.6 and 16.25 mg kg^1, with a mean of 12.67 mg kg^{-1}. From each field a representative sample of 500 g was removed. All samples were mixed thoroughly and a 500 g composite sample served as the source of bacterial isolates. Soil was maintained at 4°C for microbiological studies. Physicochemical properties of the soil were estimated by standard methods (Page *et al*. 1982) (**Table 1**).

Received: 7 February, 2011. Accepted: 3 December, 2011.

Research Note

Table 1 Important physicochemical properties of the experimental soil.

Properties	Status
pH	7.22
Electrical conductivity	1.18 ds m^{-1}
Organic carbon	9.90 g kg^{-1}
Available nitrogen	152.52 kg ha^{-1}
Available phosphorus	65.7 kg ha^{-1}
Available potassium	135.5 kg ha^{-1}
Olsen extractable arsenic	2.33 mg kg^{-1}
Total arsenic	15.68 mg kg^{-1}

Olsen extractable arsenic concentration was determined by an atomic absorption spectrophotometer (Perkin Elmer Analyst 200, USA) coupled with FIAS 400.

Isolation of arsenic-resistant bacteria

Basal salt minimal medium (BSMYI) with a pH value of 8 (Yamamura *et al.* 2003; Pattamaporn *et al.* 2008) was used for isolation of arsenic transforming bacteria. The medium was autoclaved at 121°C for 15 min. One gram of moist soil sample was dissolved in 10 ml of 0.9% NaCl and shaken for 30 min. Then 5 ml of soil suspension was inoculated into 50 ml BSMYI media containing 150 µg ml^{-1} As^{V} and incubated at 30°C on rotary shaker at 120 rpm for 2 days. 5 ml of culture was transferred into fresh BSMYI medium containing 250 µg ml^{-1} As^{V}, and transferred twice into new medium that was supplemented with 500 µg ml^{-1} As^{V}. For isolation of arsenic-resistant bacteria following the above enrichment process, 100 µl of culture was spread on BSMYI agar that contained 500 µg ml^{-1} As^{V} and incubated at 30°C for 2 days. Sodium-arsenate was filter sterilized and added to the medium as the source of As^{V}.

Arsenic tolerance capacity of the isolates

In order to assess the arsenic tolerance limit of the 20 isolated bacteria, the cultures were grown in 15 ml of BSMYI broth containing different concentration of As^{V} (1000, 2000, 4000, 6000, 8000, 10000, 12000, 14000 and 16000 mg l^{-1}) and As^{III} (50, 75, 100, 125, 150, 175, 200, 225 and 250 mg l^{-1}) in 50-ml conical flask and were incubated at 30°C for 3 days. The growth appearance of cultures was determined by turbidity measurements following spectrophotometry (optical density at 600 nm) (VARIAN CARY-50 UV-VIS).

Selection of efficient arsenic-removing bacteria

Initially 28 isolates were coded as AGH-01 to AGH-28 where 'A' stands for Arsenic and 'GH' stands for Ghentugachhi. Finally 20 pure bacterial isolates were obtained by a repeated streak plate method. As^{V} solution at 25 mg l^{-1} was added to the media. As there were 10 isolates, 10 media were prepared to which inocula were added. Besides, 10 more media were prepared where inocula were not added, the controls. In general, for any experiment only one control was used. However, as the 10 bacterial isolates needed some specific environment for each, a common control might not serve the purpose. Another 20 sets of media were prepared with As^{III} solution at 25 mg l^{-1}. Among these 20 bacterial isolates, 10 with the greatest arsenic tolerance were selected for studying their arsenic-removing ability in BSMYI broth containing 25 µg ml^{-1} of As^{V} and As^{III}. Conical flasks containing 20 ml of this broth were incubated with 500 µl of bacterial cells ($OD_{600\ nm}$ = 0.6) of each isolates and allowed to shake in a rotary shaker at 100 rpm at 30°C. After three days of incubation, broth cultures were centrifuged at 10,000 rpm for 5 min to separate the cell mass and liquid broth. Liquid medium was filtered through a 0.45 µm Millipore filter and the concentration of arsenic for each isolate was measured. Liquid medium amended with arsenic but not inoculated with bacteria and liquid media inoculated with bacteria but no arsenic were used as blanks for each set of experiments. The total arsenic content was measured by atomic adsorption spectroscopy (Perkin Elmer Analyst 200, USA) coupled with FIAS 400).

Phenotypic and biochemical characterization of the isolate

Colony characteristics of the isolates on BSMYI slants and cellular morphology by negative staining, Gram character, spore and capsule formation as well as different biochemical characteristics of the isolates were studied by following standard procedures (Holtz 1993).

Identification of the bacterial isolates based on 16S rDNA

For the molecular identification of the bacterial isolates, total genomic DNA was extracted as described by Sambrook and Russel (2001) and the 16S rRNA gene was amplified by PCR using a forward primer Y1(40) (5'–TGG CTC AGA AGG AAG GCG GCG GC–3') and a reverse primer Y2(337) (5'–CCC ACT GCT GCC TCC CGT AGG AGT–3'), commercially purchased from Chroumous Biotech Pvt. Ltd., City, India. Before amplification, DNA was initially denatured for 5 min at 94°C and then 1 min at 94°C. After amplification, a final extension step (10 min at 72°C) was performed. The cycling parameters consisted of 35 cycles at: 94°C for 5 min (initial denaturation), 94°C for 1 min (denaturation), 52°C for 1 min (primer annealing), 72°C for 5 min (final extension). The samples were maintained at 4°C until analysis by 1% agarose (Sigma) gel electrophoresis. Sequencing was carried out by Chroumous Biotech Pvt. Ltd., City, India and 16SrRNA gene sequences were subjected to BLAST-N analysis (http://www.ncbi.nlm.nih.gov/) to identify the most similar species.

RESULTS AND DISCUSSION

Isolation of arsenic-resistant bacteria and determination of arsenic tolerance capacity

Twenty pure bacterial isolates were obtained from arsenic-enriched BSMYI culture medium) followed by serial dilution pour plate technique and repeated streak plate methods. After 3 days of incubation the arsenic tolerance capacity of these isolates was estimated with different concentration of As^{V} and As^{III} in BSMYI broth (**Table 2**). It was observed that all isolates could grow and withstand the arsenic toxicity up to 8000 mg l^{-1} of As^{V} and 125 mg l^{-1} of As^{III}. Ten bacterial isolates viz. AGH-03, AGH-07, AGH-10, AGH-12, AGH-15, AGH-16, AGH-18, AGH-21, AGH-23, AGH-26 sustained and grew in an environment with the concentration of 12,000 mg l^{-1} As^{V} of and 200 mg l^{-1} of As^{III}. From the results it was observed that all bacterial isolates do not have the same tolerability to arsenic toxicity. This might be due to developing of arsenic tolerance and resistant ability of the inherent individual soil microorganisms as described by Smith (1998). These ten isolates were selected for further study. Out of these ten, a few of the isolates could have been grown up to 14,000 mg l^{-1} of As^{V} broth (AGH-03, AGH-16, AGH-21, AGH-23) and up to 225 mg l^{-1} of As^{III} broth (AGH-03, AGH-21), no growth was observed beyond the said As^{V} and As^{III} concentrations. Existence of specific mechanism in side the microbe cell along with some environmental factors made the isolates capable to resist and even grow further against the higher metal toxicity (Smith 1998). Mechanisms of resistance by microorganism include microbial surface sorption, enzymatic transformation, precipitation by oxidation/reduction reaction, and biosynthesis of metal binding proteins or extracellular polymers. Whereas environmental factors may include the surrounding pH and redox potential, metal speciation, soil particulates and soluble organic matters (Srinath 2002; Zoubilis 2004). Those isolated microorganisms might have the similar arsenic resistance mechanisms with different degrees. Minimal inhibitory concentrations of arsenic tolerant bacteria were reported by Krumova (2008) and Chang (2007) which were almost similar to these findings. The arsenic toxicity of As^{III} was almost 100 times greater than As^{V}. Similar observations have been reported by many workers (Ji and Silver 1992; Hiroki 1993; Omerland and Stolz 2005).

Table 2 Arsenic (As^V and As^{III}) tolerance capacity of the bacterial isolates after 3 days of incubation.

Strain	Concentration of As^V in mg l^{-1}									Concentration of As^{III} in mg l^{-1}								
	1000	2000	4000	6000	8000	10000	12000	14000	16000	50	75	100	125	150	175	200	225	250
AGH-01	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-
AGH-02	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-
AGH-03	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
AGH-04	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-
AGH-05	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-
AGH-06	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-
AGH-07	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	-
AGH-08	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-
AGH-09	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-
AGH-10	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	-
AGH-11	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-
AGH-12	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	-
AGH-13	+	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-
AGH-14	+	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-
AGH-15	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	-
AGH-16	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-
AGH-17	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-
AGH-18	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	-
AGH-19	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-
AGH-20	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-
AGH-21	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
AGH-22	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-
AGH-23	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-
AGH-24	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-
AGH-25	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-
AGH- 26	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	-

+ = growth; - = no growth

Arsenic removal by the isolates

Among the 10 selected isolates, AGH-21 was the most efficient one for removing both As^V (6.4 mg l^{-1}) and As^{III} (7.6 mg l^{-1}) from the broth (**Table 3**), which initially contains 25 mg l^{-1} of either As^V or As^{III}. Results show that AGH-21 is capable of removing 26% of As^V and 31% of As^{III} from the liquid media during the 3 days incubation period. On an average, the removal of arsenic ranges from 1.0-6.4 mg l^{-1} of As^V and 2.0-7.6 mg l^{-1} of As^{III}. Irrespective of form of arsenic (As^V and As^{III}), the bacterial isolates act in the same manner (**Fig. 1A, 1B**) so far the residual status of As^V and As^{III} in the BSMYI media remain after the 3 days incubation period. The removal capacity of the arsenic by the bacterial isolates, in general was greater for As^V over As^{III}. Study shows that maximum amount of As^V (23.98 mg l^{-1}) and As^{III} (22.99 mg l^{-1}) remains in the media when AGH-15 inoculum was added to the media. The same was at the lowest level (As^V 18.6 mg l^{-1} and As^{III} 17.4 mg l^{-1}) when AGH-21 inoculum was present in the media. At the end of the incubation period status of As^V and As^{III} were markedly low under AGH-21 than other isolates. The reason for the loss or removal of arsenic from the broth may possibly due to volatilization as well as microbial cell accumulation (Pornsawan and Nootra 2001; Qin 2006; Cernansky 2009). It has been reported that a number of bacterial species, namely *Proteus* sp., *Bacillus* sp., *Escherichia coli*, *Flavobacterium* sp., *Corynebacterium* sp. *Pseudomonas* sp. possess varying degrees of arsenic accumulating abilities Through biomethylation most of these species also capable of transforming more toxic inorganic form of arsenic (As^V and As^{III}) into methylated arsenic species such as MMA, DMA, TMA and TMAO, which are less toxics to humans. The isolate AGH-21 showed the higher arsenic resistance ability and able to remove maximum amount of As^V and As^{III}, was selected for identification.

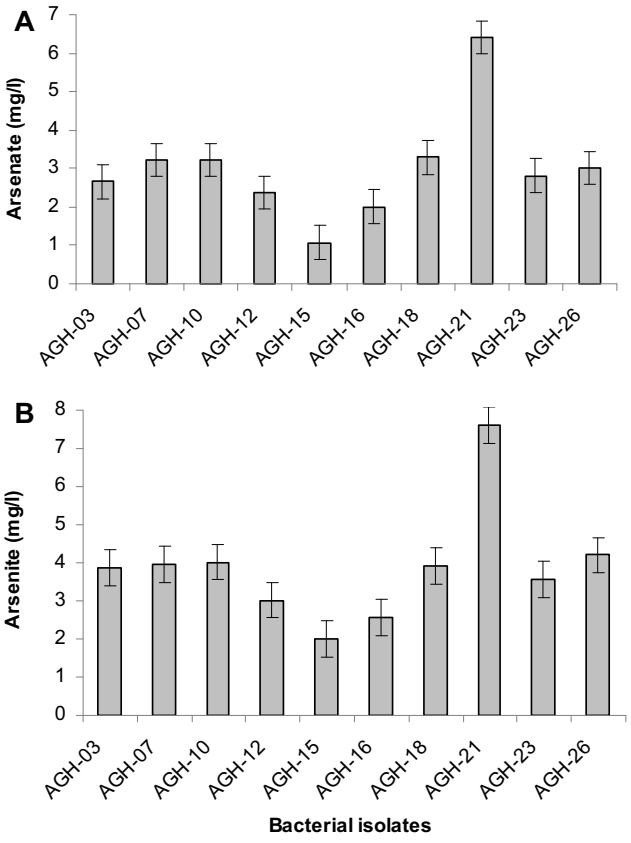

Fig. 1 Role of bacterial isolates on residual status of added As^V (arsenate; A) and As^{III} (arsenite; B) in BSMYI medium 3 days after incubation. N = 3. Error bars indicate standard error of mean (SE), and are identical.

Phenotypic and biochemical characteristics of the efficient bacterial isolate

Phenotypic characteristics of the isolated pure culture of AGH-21 showed white irregular undulating colonies. The isolate was Gram-positive, form endospore and capsule, showing long rod shaped morphology in chain under the microscope. Biochemical characteristics revealed that the isolate could utilize glucose, fructose, nitrate and could hydrolyse starch. It was catalase, oxidase and gelatinase positive but indole, methyl red and Voges-Proscure negative.

Table 3 Removal of As^{V} and As^{III} (mg l^{-1}) from BSMYI media initially contains 25 mg l^{-1} As^{V} and As^{III} after 3 day incubation at 30°C.

Bacterial isolates	Removal of As^{V} from broth (mg l^{-1})	Removal of As^{V} from broth (mg l^{-1})
AGH-03	3.20	3.10
AGH-07	4.05	3.90
AGH-10	3.73	3.22
AGH-12	2.86	2.57
AGH-15	2.62	2.37
AGH-16	1.02	2.01
AGH-18	3.61	3.21
AGH-21	6.40	7.60
AGH-23	3.31	3.42
AGH-26	4.23	4.12

The isolate showed alkaline reaction on triple sugar iron agar slant.

Identification of the bacterial isolate AGH-21 on the basis of 16S rDNA sequences

Molecular characterization of the strain based on sequencing of 16S rDNA and subsequent comparison with existing databases in GenBank, the isolate AGH-21 was identified as *Bacillus flexus* (accession number: HQ834295). Arsenic oxidizing microbes were first identified in cattle-dipping fluids in South Africa (Green1918), where a bacteria, provisionally referred to as *Bacillus arsenoxydans*, was able to grow in culture media containing up to 1% As_2O_3 as As^{III}, which brought about its oxidation to As^{V}. Several strains of *Bacillus sp* have also been recently reported to be able to oxidize As^{III} to As^{V} (Ike 2008; Chang and Kim 2010).

ACKNOWLEDGEMENTS

The authors thank Dr. Jaime A. Teixeira da Silva for significant improvements in scientific content, language and grammar.

REFERENCES

Chang JS, Kim IS (2010) Arsenite oxidation by *Bacillus* sp. strain SeaH-As22w isolated from coastal seawater in Yeosu Bay. *Environmental Engineering Research* **15 (1)**, 15-21

Green HH (1918) Isolation and description of a bacterium causing oxidation of arsenite to arsenate in cattle dipping baths. *Report on Directory of Veterinary Research for South Africa* **89**, 713-764

Guha Majumder DN (2008) Chronic arsenic toxicity and human health. *Indian Medical Research* **128**, 436-447

Hiroki M (1993) Effect of arsenic pollution on soil microbial population. *Soil Science and Plant Nutrition* **39**, 227-235

Holtz JD (1993) *Bergey's Manual of Determinative Bacteriology* (9th Edn), Williams and Wilkins, Baltimore

Ike M, Miyazaki T, Yamamoto N, Sei K, Soda S (2008) Removal of arsenic from groundwater by arsenite-oxidizing bacteria. *Water Science and Technology* **58 (5)**, 1095-1100

Ji G, Silver X (1992) Reduction of arsenate to arsenite by ArsC protein of the arsenic resistance operon of *Staphylococcus aureus* plasmid p1258. *Proceedings of the National Academy of Sciences USA* **89**, 9474-9478

Krumova K, Nikolovska M, Groudeva V (2008) Isolation and identification of arsenic-transforming bacteria from arsenic contaminated sites in Bulgaria. *Biotechnology and Biotechnological Equipment* **22**, 721-728

Meharg AA, Williams PN, Adamako E, Lawgali YY, Deacon C, Villada A, Cambell RCJ, Sun GX, Zhu YG, Feldmann J, Raab A, Zhao FJ, Islam R, Hossain S, Yanai J (2009) Geographical variation in total and inorganic arsenic content of polished (white) rice. *Environmental Science and Technology* **43**, 1612-1617

Mukhopadhyay D, Sanyal SK (2004) Complexation and release isotherm of arsenic in arsenic-humic/fulvic equilibrium study. *Australian Journal of Soil Research* **42**, 815-824

Omerland RS, Stolz JF (2005) Arsenic, microbes and contaminated aquifers. *Trends in Microbiology* **13 (2)**, 45-49

Page AL, Miller RH, Keeney DR (1982) *Methods of Soil Analysis* (Part 2, 2nd Edn), Soil Science Society of American Journal Madison, Wisconsin, USA

Pattamaporn A, Poonsuk P, Vorasan S (2008) Isolation of arsenic-tolerant bacteria from arsenic-contaminated soil. *Songklanakarin Journal of Science and Technology* **30**, 95-102

Pornsawan V, Nootra P (2001) Selection of fungi capable of removing toxic arsenic compounds from liquid medium. *Science Asia* **27**, 83-92

Quin J, Rosen BP, Zhang Y, Wang G-J, Franke S, Rensing C (2006) Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite *S*-adenosylmethionine methyltrasferase. *Proceedings of the National Academy of Sciences USA* **103**, 2075-2080

Rehman A, Butt AS, Hasnain S (2010) Isolation and characterization of arsenite oxidizing *Pseudomonas* lubricants and its potential use in bioremediation of wastewater. *African Journal of Biotechnology* **9**, 1493-1498

Sambrook J, Russel DW (2001) *Molecular Cloning: A Laboratory Manual* (3rd Edn), CSH Laboratory Press, Cold Spring Harbor, New York

Cernansky S, Kolensik M, Sevc J, Urik M, Hiller E (2009) Fungal volatilization of trivalent and pentavalent arsec under laboratory condition. *Bioresource Technology* **100**, 1037-1040

Smith AH, Goycolea M, Haque R, Biggs ML (1998) Marked increase in bladder and lung cancer mortality in a region of northern Chile due to arsenic in drinking water. *American Journal of Epidemiology* **147**, 660-669

Srinath T, Verma T, Ramteke PW, Garg SK (2002) Chromium (VI) biosorption and bioaccumulation by chromate-resistant bacteria. *Chemosphere* **48**, 427-435

World Health Organization (WHO) (1996) *Guidelines for Drinking Water Quality* (2nd Edn), *Health Criteria and Other Supporting Information* (Vol 2), Geneva

Yamamura S, Ike M, Fujita M (2003) Dissimilatory arsenate reduction by a facultative anaerobe, *Bacillus* sp. strain SF-1. *Journal of Bioscience and Bioengineering* **96**, 454-460

Zouboulis AI, Loukidou MX, Matis KA (2004) Biosorption of toxic metals from aqueous solutions by bacteria strains isolated from metal-polluted soils. *Process Biochemistry* **39**, 1-8