Archives of Environmental Protection
Vol. 42 no. 3 pp. 58–66

PL ISSN 2083-4772
DOI 10.1515/aep-2016-0027

DE GRUYTER OPEN

Identifying an efficient bacterial species and its genetic erosion for arsenic bioremediation of gold mining soil

Lamyai Neeratanaphan[1,2]*, Tawatchai Tanee[3], Alongklod Tanomtong[1,4], Bundit Tengjaroenkul[1,5]

[1]Research Group on Toxic Substances in Livestock and Aquatic Animals
Khon Kaen University, Thailand
[2]Faculty of Science, Department of Environmental Science
Khon Kaen University, Thailand
[3]Faculty of Environment and Resource Studies
Mahasarakham University, Thailand
[4]Faculty of Science, Department of Biology
Khon Kaen University, Thailand
[5]Faculty of Veterinary Medicine, Department of Veterinary Medicine
Khon Kaen University, Thailand

*Corresponding author's e-mail: hlamya@kku.ac.th

Keywords: arsenic-polluted soil, gold mining soil, efficient bacteria, genetic erosion.

Abstract: To improve bioremediation of arsenic (As) contamination in soil, the use of microorganisms to efficiently reduce As and their assessment of genetic erosion by DNA damage using genomic template stability (GTS) evaluation and using RAPD markers were investigated. The five sites examined for microorganisms and contaminated soils were collected from affected gold mining areas. The highest As concentration in gold mining soil is 0.72 mg/kg. Microorganism strains isolated from the gold mining soil samples were tested for As removal capacity. Two bacterial isolates were identified by 16S rRNA gene sequence analysis and morphological characteristics as *Brevibacillus reuszeri* and *Rhodococcus* sp. The ability to treat As in nutrient agar (NA) at 1,600 mg/L and contaminated soil samples at 0.72 mg/kg was measured at 168 h, revealing more efficient As removal by *B. reuszeri* than *Rhodococcus* sp. (96.67% and 94.17%, respectively). Both species have the capacity to remove As, but *B. reuszeri* shows improved growth compared to the *Rhodococcus* sp. *B. reuszeri* might be suitable for adaptation and use in As treatment. The results are in agreement with their genetic erosion values, with *B. reuszeri* showing very little genetic erosion (12.46%) of culture in As concentrations as high as 1,600 mg/L, whereas 82.54% genetic erosion occurred in the *Rhodococcus* sp., suggesting that *Rhodococcus* sp. would not survive at this level of genetic erosion. Therefore, *B. reuszeri* has a high efficiency and can be used for soil As treatment, as it is capable to tolerate a concentration of 0.72 mg/kg and as high as 1,600 mg/L in NA.

Introduction

Metal accumulation by solid substances can counteract metal mobilization in the environment if the solid substance is immobile. Arsenic (As) is an extremely toxic metalloid widely distributed in soil and water. The toxicity and mobility of As in the environment is dependent on the chemical form or species in which it exists. It is well known that inorganic As, such as arsenite and arsenate, are the most toxic As species. Additional anthropogenic source of mining activities, utilization of As-based pesticides or herbicides, and irrigation with As-contaminated water causes heavy contamination of soil, especially in farmland ecosystems (Jackson et al. 2006, Chen et al. 2008, Williams et al. 2009). Moreover, As is also commonly associated with sulfides, such as in sulfidic ore deposits. Other natural sources of As include volcanic activities, windborne soil particles, sea salt sprays and microbial volatilization of As (Frankenberger and Arshad 2002). As is a major contaminant of gold mining activities because the concentrations in the soil found to be high, polluting the environment hence causing environmental health problems. The concentrations of As in non-contaminated soils are typically well below 10 mg/kg. Its presence at elevated concentrations in soils

is due to both anthropogenic and natural inputs (Adriano 2001). In addition, As is known to have mutagenic and genotoxic effects on humans, and it has been associated with an increased risk of skin, kidney, lung, and bladder cancers (Shahedur et al. 2014). Cleaning the environment by removing these persistent and hazardous contaminants requires effective approaches allowing for precise restoration of polluted sites. Physico-chemical methods for remediation of metals from soils involve chemical extraction with acids or chelating agents, electrolysis, or size separation of the fraction of soil particles with the highest metal content (Page and Page 2002). Contamination of soils with heavy metals and metalloids, such as lead, cadmium, and arsenic, among others, represents a serious threat for the ecosystem and human health and requires the implementation of appropriate remedial measures. Microbial based technologies for metal extraction have become attractive because they are cost effective compared to chemical methods and might be applicable for a large number of inorganic pollutants (Hutchins et al. 1986). Bioremediation of As-contaminated soils has recently gained importance, in part because the processes promoting the bioremediation and biomethylation of As are still poorly understood. In addition, the bioremediation of As by microorganisms has been widely applauded because of the potential advantages of providing a cost-effective technology and an environmentally friendly method for heavy-metal removal (Valls and Lorenzo 2002). The removal of As from contaminated soils by applying anaerobic bioremediation techniques has been recently investigated by Chatain et al. (2005) and Ignatiadis and Battaglia-Brunet (2005).

There are several molecular techniques used to identify the genetic relationships and species of microorganisms. One of the most popular and efficient techniques is the sequence alignment of 16S rRNA genes, which are highly conserved regions among an identical species and genus (Clarridge 2004, Neeratanaphan et al. 2015). This sequence can be successfully used for analysis of genetic diversity and species identification in bacteria (Gremion et al. 2003, Lindh et al. 2005, Flynn et al. 2013, Kang et al. 2013), including the evaluation of bacterial--heavy metal relatedness (Kozdrój and Van Elsas 2000, Park et al. 2006).

The gold mining industry has encountered problems with As for many years, because it is generated during gold extraction when arsenopyrite is broken down and it also and diffuses into soil and water, polluting the environment (Henke 2009). It can be distributed in either soil or water and transported to other places, polluting water resources and subsequently affecting water for daily consumption. Thus, organisms in the area near gold mines will adapt to their environment. Thailand has a gold mine located in the Wangsaphung district, Loei province, which affects the humans living in close proximity. Microorganisms are a suitable model to study possible impacts on human health if they are eukaryotes, allowing the examination of genetic erosion or genotoxicity.

A genotoxicity measurement caused by heavy metals in living things, including microorganisms, is mainly related to sensitivity and short response time (Gupta and Sarin 2009). The released pollutants can cause morbidity and mortality in the exposed organisms, possibly motivating order changes such as alterations to population dynamics and changes to biological diversity (An et al. 2012). The genotoxic effects depend on the oxidative state of the metal, its concentration and the duration of its exposure. In general, effects are more pronounced at higher concentrations and at longer exposure durations (Bhowmik 2000). Various molecular approaches, such as DNA fingerprinting based on inter simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) methods are generally used to effectively identify genetic relationships. The banding patterns can be scored for genomic template stability (GTS) evaluation to detect various types of previously identified DNA damage and mutations in bacteria, plants and animals (Neeratanaphan et al. 2014a, Neeratanaphan et al. 2014b). Gupta and Sarin (2009) used RAPD bands for GTS evaluation in *Hydrilla verticillata* and *Ceratophyllum demersum* and treated it with Cd, Hg and Cu to demonstrate DNA damage. Zhou (2011) also used RAPD bands for GTS evaluation, identifying DNA damage in *Euplotes svannus* (Protozoa, Ciliophora) induced by nitrofurazone in marine ciliates.

In this work, the authors aim to study As contamination level in soil and to identify the species of microorganisms present in the soil and their effectiveness in As treatment for further use near gold mining areas. Additionally, genetic erosion in microorganisms impacted by As was evaluated by assessing DNA damage and inverting the GTS evaluation by studying a different RAPD marker.

Materials and Methods

Sampling sites and As soil contamination measurements

The five sampling sites are located in Wangsaphung district, Loei province of Thailand near the gold mine and are defined as the affected area, (Figure 1). As concentrations in the soil samples were determined with an atomic absorption spectrometry (AAS) model Analyst 300, Perkin-Elmer (Correira et al. 2003).

Microorganism screening and species identification

The microorganism strains were isolated from the soil sample observed to have the highest As concentration (0.72 mg/kg) at site 1. The medium used for isolation was nutrient agar (NA) (0.5% peptone, 0.5% NaCl, 0.2% yeast extract, 0.2% beef extract and 1.5% agar, pH 7.0, all *W*/*V*) containing 10 mg/L of sodium arsenate (Suresh et al. 2004). The microorganisms isolated included both fungi and bacteria. The fungi and bacteria obtained from the screening exercise were tested to determine whether fungi or bacteria were more As resistant in NA supplemented with 10, 100, 500, 1,000, 1,200, 1,400, 1,600 and 1,800 mg/L of As, respectively. Finally, two bacteria isolates, B109 and B204, were observed to be more tolerant than fungi. Both isolates were identified by 16S rRNA gene sequencing with dendrogram construction using MEGA5 program (Tamura et al. 2011) and genetic similarity values confirmed at the Collaborative Unit of Biological Science and Biotechnology of Mahidol University and Osaka University. Additionally, morphological characteristics were examined by scanning electron microscopy (SEM) using a LEO 1450VP. Specimens were prepared by fixation, dehydration and coating with a very thin layer of carbon (Lyman et al. 1990).

Testing of As remediation by Brevibacillus reuszeri (B109) and Rhodococcus sp. (B204)

The As concentrations were selected from a reference noting that the highest As values found in water are 300 mg/L (Groundwater Research Center 2010), and the two bacteria were cultured in petri dishes containing nutrient broth (NB) contaminated with As at 300 mg/L and incubated at 37°C. The remaining As in the NB were measured at 0, 24, 48, 72, 96, 120, 144 and 168 h of culture using AAS detection in triplicate (Correira et al. 2003).

Bioremediation of As in Gold Mining Soils

In gold mining soils, As can be found at concentrations as high as 0.72 mg/kg. Therefore, this concentration was selected for As remediation by the isolated bacteria at 0, 24, 48, 72, 96, 120, 144 and 168 h. After As remediation, the remaining concentrations of As in the soil samples were measured by AAS, and the growth of bacteria in the soil samples was detected with the PEG-DOG method (Trung et al. 2011).

RAPD marker, GTS evaluation and genetic erosion

The bacteria were cultured in NA supplemented with As concentration levels of 0, 100, 500, 1,000 and 1,600 mg/L for 168 h. DNA was extracted from bacterial cells using the Genomic DNA Extraction Kit (RBC Bioscience, Taiwan) following the manufacturer's instructions. The extracted DNA was assessed by 0.8% agarose gel electrophoresis and diluted to a final concentration of 20 ng/µl. Two replicate experiments were performed by DNA extraction, RAPD banding patterns and analysis of DNA fingerprint profiles for GTS evaluation. Genetic erosion was converted from the GTS value.

Amplifications were performed on each sample in 25 µl reactions containing GoTaq Green Master Mix (Promega), 0.5 µM primers and 5 ng DNA templates. Fifty RAPD primers were screened, and the successful primers are listed in Table 1. The reaction mixture was pre-denatured at 94°C for 3 min, and the amplification was performed with the following 35 thermal cycles: denaturation for 1 min at 94°C, annealing for 45 sec at 40°C, extension for 2 min at 72°C; followed by a final extension for 7 min at 72°C using a Swift™ Maxi Thermal Cycler (Esco Micro Pte. Ltd.). Amplification products were detected by 1.2% agarose gel electrophoresis in TAE buffer and visualized using ethidium bromide staining. The resulted RAPD bands were used for dendrogram construction.

The RAPD bands from the successful amplifications were documented as diallelic characters: present = 1 and absent = 0. These banding data were used for GTS evaluation by percentages. The equation GTS = 100 − (100 × *a*/*n*) was used to calculate this value, in which *a* is RAPD changes detected in each sample treated and *n* is the number of total bands in the control (Atienzar et al. 1999). Genetic erosion was calculated from the GTS value.

Results

As soil contamination measurements at the five sampling sites

The concentrations of As in soils from the five sample sites ranged from the lowest, 0.04 mg/kg, at site 5 to the highest, 0.72 mg/kg, at site 1. The average concentrations of As detected from three replicates at sites 1–5 were 0.72±0.01, 0.36±0.01, 0.57±0.01, 0.30±0.06 and 0.04±0.01 mg/kg, respectively, as shown in Table 2.

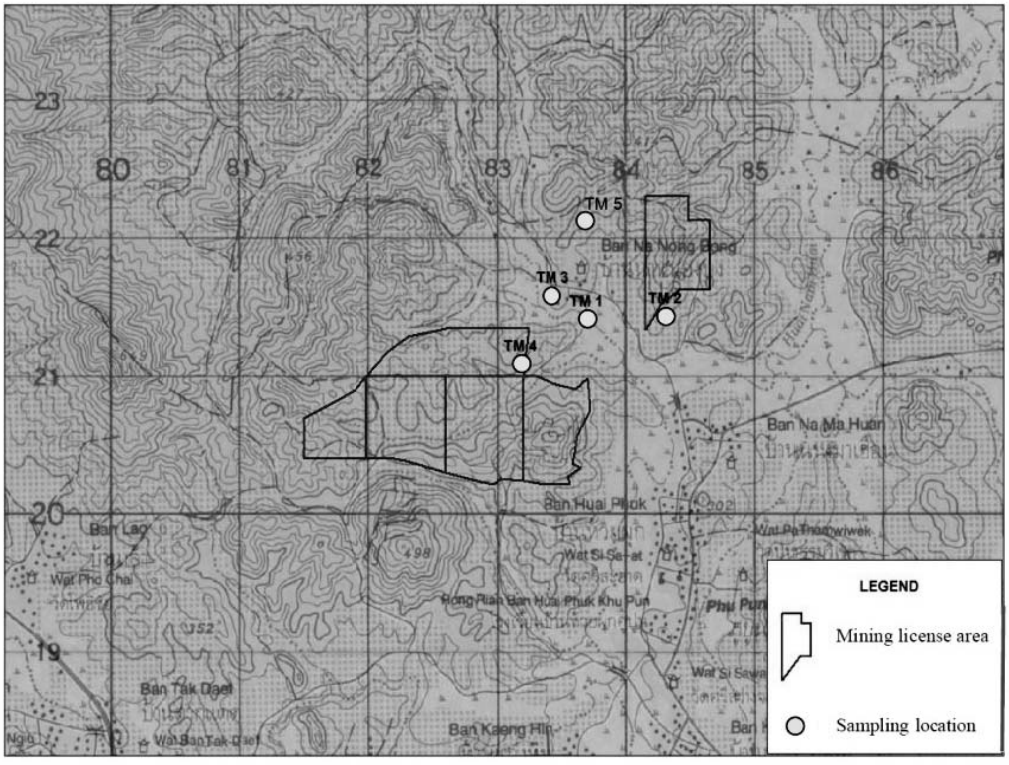

Fig. 1. An overview of the gold mining area and locations of the five sampling sites as shown by numbers 1 to 5 (Groundwater Research Center 2010)

Table 1. Successful primer sequences for RAPD fingerprinting of the two bacteria isolates *B. reuszeri* (B109) and *Rhodococcus* sp. (B204)

Primer	Nucleotide sequence (5′–3′)	Successful for		Primer	Nucleotide sequence (5′–3′)	Successful for	
		B109	B204			B109	B204
RA01	CAGGCCCTTC	✓	–	RB16	TTTGCCCGGA	✓	–
RA02	TGCCGAGCTG	✓	✓	RB17	AGGGAACGAG	✓	✓
RA03	AGTCAGCCAC	✓	✓	RB20	GGACCCTTAC	–	✓
RA04	AATCGGGCTG	✓	✓	RC01	TTCGAGCCAG	✓	✓
RA05	AGGGGTCTTG	✓	–	RC02	GTGAGGCGTC	✓	–
RA07	GAAACGGGTG	✓	✓	RC03	GGGGGTCTTT	–	✓
RA09	GGGTAACGCC	✓	✓	RC04	CCGCATCTAC	✓	–
RA11	CAATCGCCGT	✓	✓	RC05	GATGACCGCC	✓	✓
RA13	CAGCACCCAC	✓	✓	RC06	GAACGGACTC	–	✓
RA14	TCTGTGCTGG	✓	✓	RC08	TGGACCGGTG	–	✓
RA18	AGGTGACCGT	✓	–	RC10	TGTCTGGGTG	–	✓
RA20	GTTGCGATCC	✓	✓	RC20	ACTTCGCCAC	✓	–
RB01	GTTTCGCTCC	–	✓	RD01	ACCGCGAAGG	✓	–
RB06	TGCTCTGCCC	–	✓	RD02	GGACCCAACC	✓	–
RB07	GGTGACGCAG	–	✓	RD03	GTCGCCGTCA	✓	–
RB08	GTCCACACGG	✓	✓	RD04	TCTGGTGAGG	✓	–
RB10	CTGCTGGGAC	✓	✓	RD11	AGCGCCATTG	✓	–
RB11	GTAGACCCGT	✓	–	RD12	CACCGTATCC	✓	–
RB13	TTCCCCCGCT	–	✓	RD14	CTTCCCCAAG	✓	–
RB14	TCCGCTCTGG	–	✓	RD18	GAGAGCCAAC	✓	–
RB13	TTCCCCCGCT	✓	–	RD20	ACCCGGTCAC	✓	–
RB15	GGAGGGTGTT	✓	–	RE02	GGTGCGGGAA	✓	–

Table 2. As contamination in gold mining soil (Mean± SD)

Sampling sites	As concentrations (mg/kg)
Site 1	0.72±0.01
Site 2	0.36±0.01
Site 3	0.57±0.01
Site 4	0.30±0.06
Site 5	0.04±0.01

Microorganism screening and species identification

Colonies from the strain B109 were cream coloured, raised, smooth, and convex on nutrient agar, and B204 colonies were cream coloured, circular, and convex on nutrient agar. The photos of the strains B109 and B204 from SEM revealed the rod and coccus morphology, respectively, as shown in Figure 2. Sequence alignment and dendrogram construction from 16S rRNA gene sequence analysis showed the genetic relationship of these two strains compared to other bacteria from GenBank of the National Center for Biotechnology Information (NCBI). The isolates B109 and B204 showed genetic distances of

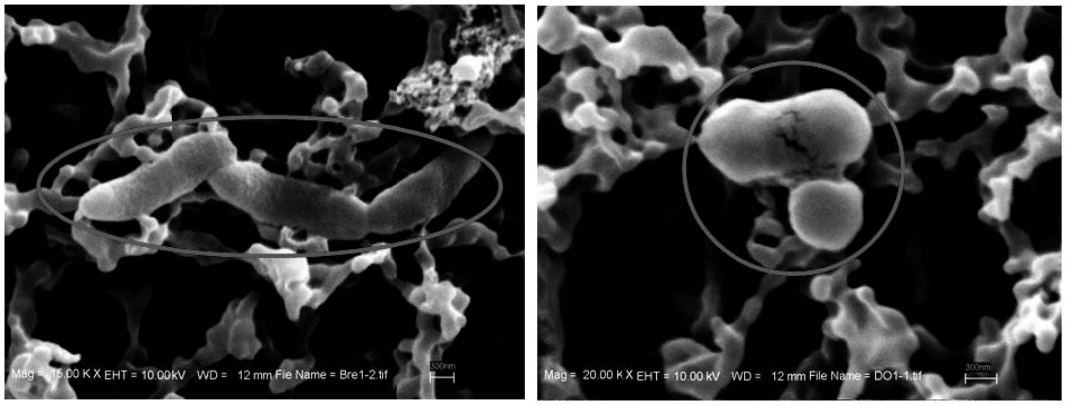

Fig. 2. Shapes of bacterial isolates B109 (A) and B204 (B) from scanning electron microscopy (SEM)

0.005 compared to *Brevibacillus reuszeri* and 0.000 compared to *Rhodococcus erythropolis*, *R. zopfii*, *R. kunmingensis*, *R. pyridinivorans*, *R. gordoniae*, *R. rhodochrous* and *R. equi*. The dendrogram separated B109 from *B. reuszeri* as shown in Figure 3 and included B204 in the branch of *Rhodococcus erythropolis*, *R. zopfii*, *R. kunmingensis*, *R. pyridinivorans*, *R. gordoniae*, *R. rhodochrous* and *R. equi* as shown in Figure 4.

Testing of As remediation by Brevibacillus reuszeri (B109) and Rhodococcus sp. (B204) in NB

Percentage of As removal by *B. reuszeri* was higher than by *Rhodococcus* sp. as shown in Table 3. Additionally, isolate B109 showed the fastest of As reduction in the NB from 24 h tight to 168 h from initial concentration of 300 mg/L as shown in Table 3 and Figure 5.

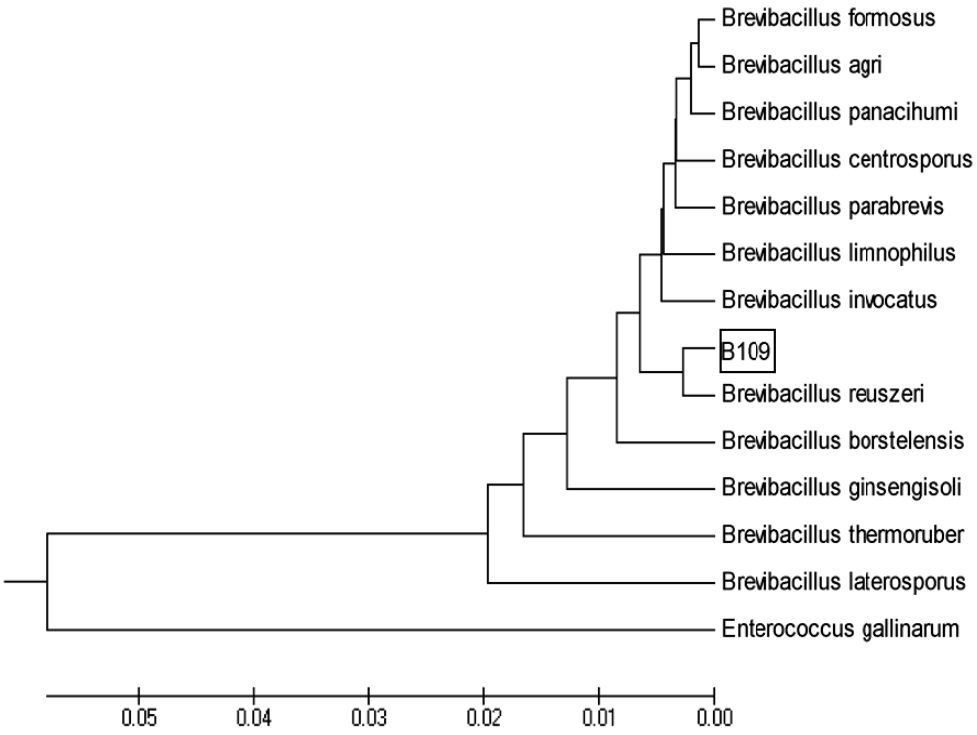

Fig. 3. The dendrogram constructed from 16S rRNA gene sequence analysis using the MEGA5 program (Tamura et al. 2011) showing the genetic relationship of isolate B109 compared to other bacteria in GenBank

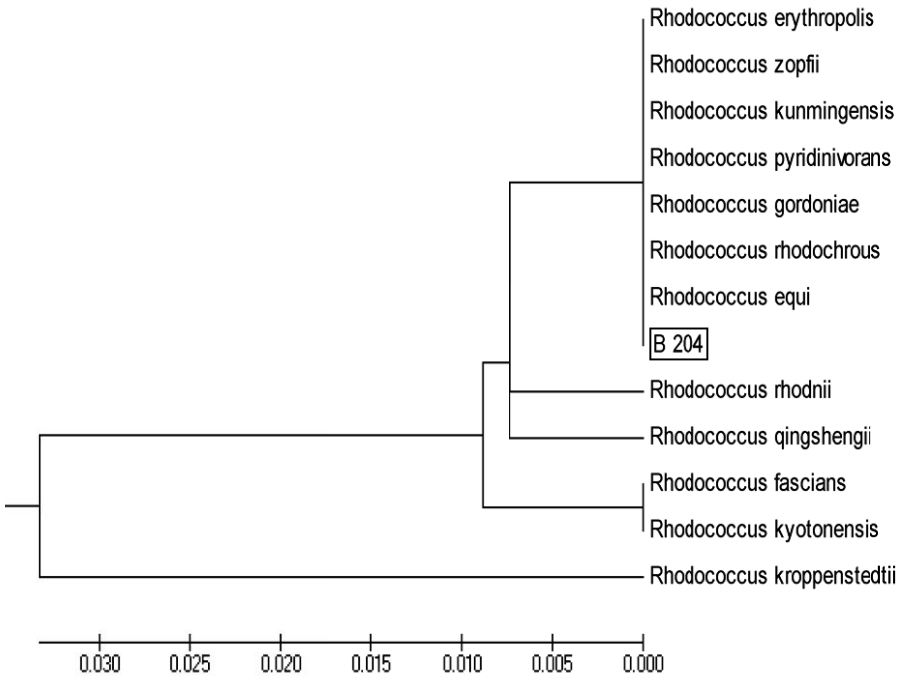

Fig. 4. The dendrogram constructed from 16S rRNA gene sequence analysis using the MEGA5 program (Tamura et al. 2011) showing the genetic relationship of isolate B109 compared to other bacteria in GenBank

Bioremediation of As in gold mining soils

Further remediation study using actual gold mining soil revealed that isolate B109 was superior to isolate B204 with more and faster removal as shown in Table 4 and Figure 6. Isolate B109 removed 96.67% of As from initial concentration of 0.72±0.01 to 0.03±0.00 mg/kg in 168 h. The concentration of As remediated by B109 after 168 h was below the level found in typical non contaminated soil of 10 mg/kg.

The growth of the two bacteria after As remediation is shown in Figure 7. *B. reuszeri* has a larger capacity for As treatment than *Rhodococcus* sp., because the residual As in soil samples treated by *B. reuszeri* was less than in *Rhodococcus* sp. When the growth measurements were performed, the number of *Rhodococcus* sp. was higher than *B. reuszeri* during hours 0–144, whereas after 144–168 h of incubation, the number of *B. reuszeri* bacteria was much higher than the *Rhodococcus* sp. These results suggest that *B. reuszeri* could be adapted for As treatment.

Table 3. The concentrations of total As in NB after treatment by *B. reuszeri* (B109) and *Rhodococcus* sp. (B204) and the percentage of remediation (Mean± SD)

Time (h)	*B. reuszeri* (B109)		*Rhodococcus* sp. (B204)	
	As remaining in NB (mg/L)	% As removal	As remaining in NB (mg/L)	% As removal
0	300	0	300	0
24	136.81±0.07	54.40	243.74±0.10	18.76
48	132.51±0.50	56.00	222.05±0.07	24.32
72	123.03±0.21	58.99	187.87±0.02	37.38
96	112.53±0.01	62.49	141.06±0.02	52.98
120	110.26±0.06	63.25	132.18±0.03	55.94
144	98.72±0.15	67.10	123.21±0.01	58.93
168	97.37±0.02	67.55	117.96±0.01	60.68

Table 4. The concentration of total As in gold mining soil samples after treatment by *B. reuszeri* (B109) and *Rhodococcus* sp. (B204) (Mean± SD)

Time (h)	*B. reuszeri* (B109)		*Rhodococcus* sp. (B204)	
	As remaining in soil sample (mg/kg)	% As removal	As remaining in soil sample (mg/kg)	% As removal
Control	0.72±0.01	0	0.72±0.01	0
0	0.61±0.01	15.28	0.70±0.02	2.78
24	0.23±0.01	68.10	0.37±0.02	48.62
48	0.15±0.00	79.17	0.22±0.01	69.45
72	0.07±0.00	90.69	0.18±0.01	75.00
96	0.06±0.01	91.67	0.14±0.01	80.56
120	0.04±0.00	94.45	0.08±0.00	88.89
144	0.04±0.00	95.14	0.05±0.01	93.06
168	0.03±0.00	96.67	0.04±0.00	94.17

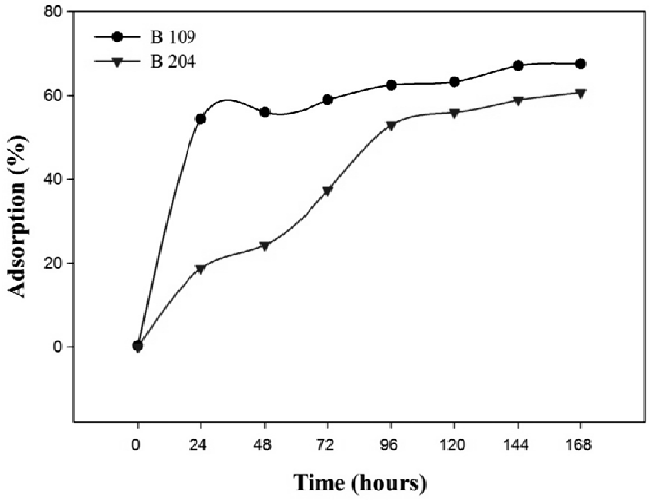

Fig. 5. As removal by *B. reuszeri* (B109) and *Rhodococcus* sp. (B204) in NB with an As concentration of 300 mg/L at 0, 24, 48, 72, 96, 120, 144 and 168 h of incubation

Assessment of DNA changes in B. reuszeri and Rhodococcus sp.

The bacteria *B. reuszeri* and *Rhodococcus* sp. were cultured in NA containing As concentrations of 0, 100, 500, 1,000 and 1,600 mg/L for 168 h. Both species were analyzed for DNA changes by GTS evaluation using RAPD markers. The 34 and 24 successful RAPD primers generated clear and analyzable fingerprinting profiles for *B. reuszeri* and *Rhodococcus* sp., respectively, and examples are shown in Figure 8. Only bands present in both samples for each experiment were included, and an identical number of 365 characteristics that were used for GTS evaluation. The GTS values are shown in Table 5 for *B. reuszeri* and *Rhodococcus* sp. The genetic erosion of the two bacteria are 0, 7.48, 6.33, 7.17, and 12.46 for *B. reuszeri* and 0, 65.08, 73.02, 69.05 and 82.54 for *Rhodococcus* sp. after exposure to As concentration of 0, 100, 500, 1,000 and 1,600 mg/L, respectively. The values of genetic erosion and As concentration levels are correlated, such that when the As concentration was high, a large amount of genetic erosion occurred.

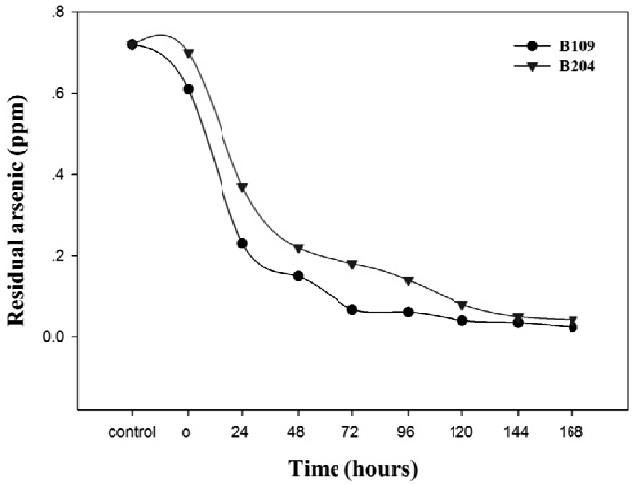

Fig. 6. Residual As in gold mining soil samples after treatment by *B. reuszeri* (B109) and *Rhodococcus* sp. (B204) after 0, 24, 48, 72, 96, 120, 144 and 168 h

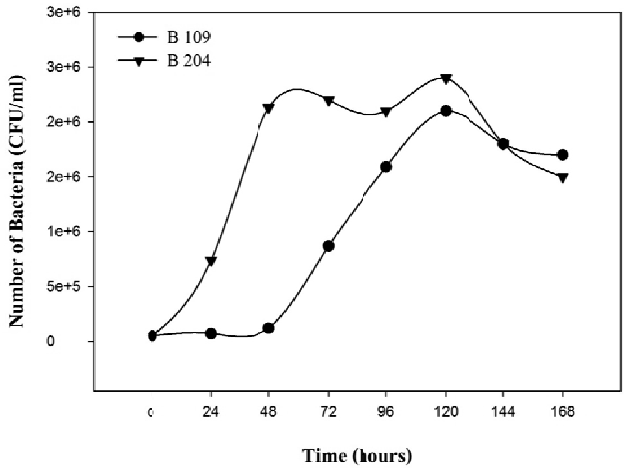

Fig. 7. Number of *B. reuszeri* (B109) and *Rhodococcus* sp. (B204) in gold mining soil samples of As treatment after 0, 24, 48, 72, 96, 120, 144 and 168 h

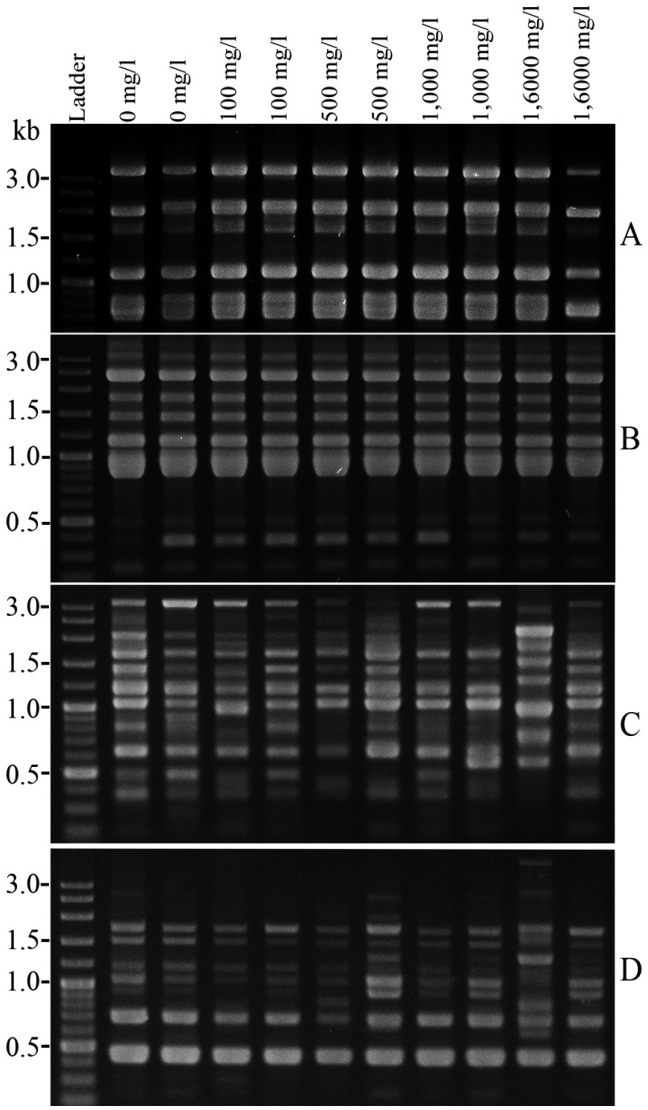

Fig. 8. Examples of RAPD banding patterns for As-treated bacteria from the primers CAGGCCCTTC (A) and CAATCGCCGT (B) for *B. reuszeri*, and AATCGGGCTG (C) and CAGCACCCAC (D) for *Rhodococcus* sp.

Table 5. Genomic template stability (GTS) and genetic erosion evaluated from the RAPD banding patterns of the bacteria *B. reuszeri* (B109) and *Rhodococcus* sp. (B204) exposed to different concentration of As

As concentration (mg/L)	GTS (%) of B109	Genetic erosion of B109	GTS (%) of B204	Genetic erosion of B204
0	100.00	0	100.00	0
100	92.52	7.48	34.92	65.08
500	93.77	6.33	26.98	73.02
1,000	92.83	7.17	30.95	69.05
1,600	87.54	12.46	17.46	82.54

Discussion

It is well known that microorganisms can be used to remove heavy metals. Microorganisms have been widely applauded because of the potential advantages of providing a cost--effective technology and an environmentally friendly method for heavy-metal removal (Valls and Lorenzo 2002). The removal of As from contaminated soils by applying anaerobic bioremediation techniques has been recently investigated by Chatain et al. (2005) and Ignatiadis and Battaglia-Brunet (2005). The problem of As contamination occurs in soil where majority of gold mine tailings pond sediment

(Anderson and Cook 2004). Therefore, the authors screened for microorganisms to efficiently treat As contamination in areas near a gold mine that is defined as an affected area in Thailand. Microorganism screening was performed in soil samples from the hazardous areas, called sites 1–5, and two groups, fungi and bacteria, were observed. When the fungi and bacteria were tested in NA to identify which has a higher capacity for As removal, two bacteria isolates were selected and called B109 and B204. Sequence analysis of 16S rRNA is frequently used for bacterial identification and has been widely reported (Gremion et al. 2003, Lindh et al. 2005, Kang et al. 2013). For the B109 16S rRNA, the genetic distance was 0.005 compared to *Brevibacillus reuszeri* in the dendrogram and showed a high similarity of 0.995, so this isolate is likely to be *B. reuszeri*. For B204, there is no genetic distance between the seven species *Rhodococcus erythropolis*, *R. zopfii*, *R. kunmingensis*, *R. pyridinivorans*, *R. gordoniae*, *R. rhodochrous* and *R. equi* above. Therefore, it cannot be identified as a single species, but only as the genus *Rhodococcus* sp. When all of the data were examined, including the morphological characteristics assessed by SEM photos and 16S rRNA sequence, these isolates were concluded to be *B. reuszeri* and *Rhodococcus* sp.

When testing As remediation by *B. reuszeri* and *Rhodococcus* sp., the results showed that both species possess an ability for As treatment. *B. reuszeri* can remove 54.40–67.55% As over 24–168 h, and *Rhodococcus* sp. can remove 18.76–60.68% As over 24–168 h from a starting As concentration of 300 mg/L in NB. The experiment indicated more As removal after a long incubation of 168 h. After this treatment, soil contaminated with As by gold mining was used to examine As removal in an environmentally relevant situation with a starting As concentration of 0.72 mg/kg. The As percentages after treatment were 15.28–96.17% over 24–168 h by *B. reuszeri* and 2.78–94.17% over the same time period by *Rhodococcus* sp. Similar to culturing in NB, after a long incubation, the amount of As removed by both *B. reuszeri* and *Rhodococcus* sp. was higher, as shown in Tables 3 and 4. However, when tested for growth under the same As concentration of 0.72 mg/kg, *B. reuszeri* grew well, as shown by the graph in Figure 7, and from the time period of 144–168 h, the number of *B. reuszeri* bacteria was much higher than *Rhodococcus* sp. This indicated that *B. reuszeri* could be adapted and used for As treatment. The results are in agreement with the genetic erosion values, because *B. reuszeri* showed little genetic erosion at 12.46% after 168 h and in an As concentration of 1,600 mg/L, whereas 82.54% genetic erosion occurred in the *Rhodococcus* sp. The *Rhodococcus* sp. would not survive this level of genetic erosion. Therefore, *B. reuszeri* has a high efficiency and can be used for soil As treatment, with a concentration tolerance of 0.72 mg/kg and as high as 1,600 mg/L in NA.

Bioremediation of As by microorganisms has been widely accepted because of the potential advantage of providing a cost-effective and environmentally friendly technology to remove heavy metals (Valls and Lorenzo 2002). The discovery of a new As treatment species, *B. reuszeri*, might be useful to research and ameliorate hazardous As contaminated areas worldwide, because its efficiency is higher than the concentration standard levels determined at 0.40 mg/kg by The US EPA Regional Screening Level; RSL (US EPA 2002).

Conclusions

Microorganism strains isolated from the gold mining soil samples were tested for As removal capacity. Two bacterial isolates were identified by 16S rRNA gene sequence analysis and morphological characteristics as *Brevibacillus reuszeri* and *Rhodococcus* sp. Both species have the capacity to remove As, but *B. reuszeri* shows improved growth compared to *Rhodococcus* sp. Thus, *B. reuszeri* might be suitable for adaptation and use in As treatment. The results are in agreement with their genetic erosion values, with *B. reuszeri* showing very little genetic erosion (12.46%) of culture in As concentrations as high as 1,600 mg/L, whereas 82.54% genetic erosion occurred in the *Rhodococcus* sp., suggesting that *Rhodococcus* sp. would not survive this level of genetic erosion. Therefore, *B. reuszeri* has a high efficiency and can be used for soil As treatment, being able to tolerate a concentration of 0.72 mg/kg and as high as 1,600 mg/L in NA. In addition *B. reuszeri*, might be useful to research and ameliorate hazardous As contaminated areas worldwide.

Acknowledgments

This research was funded by the Research Group on Toxic Substances in Livestock and Aquatic Animals.

References

Adriano, D.C. (2001). *Trace elements in the terrestrial environment*, Springer, New York 2001.

Anderson, C.R. & Cook, G.M. (2004). Isolation and characterization of arsenate-reducing bacteria from arsenic-contaminated sites in New Zealand, *Current Microbiology*, 48, 5, pp. 341–347.

An, L.H., Zheng, B.H., Wang, L.J., Zhang, Y.Q., Chen, H., Zhao, X.R., Zhang, L. & Lei, K. (2012). Biomarker responses and genotoxicity in the mud snail (Bullacta exarata) as indicators of coastal contamination, *Marine Pollution Bulletin*, 64, 2, pp. 303–309.

Atienzar, F.A., Conradi, M., Evenden, A.J., Jha, A.N. & Depledge, M.H. (1999). Qualitative assessment of genotoxicity using random amplified polymorphic DNA: comparison of genomic template stability with key fitness parameters in *Daphnia magna* expose to benzo[α] pyrene, *Environmental Toxicology and Chemistry*, 18, 10, pp. 2275–2282.

Bhowmik, N. (2000). *Cytotoxic effects of lead compounds on plant systems*, PhD thesis, University of Calcutta, Calcutta 2000.

Chatain, V., Bayard, R., Sanchez, F., Moszkowicz, P. & Gourdon, R. (2005). Effect of indigenous bacterial activity on arsenic mobilization under anaerobic conditions, *Environment International*, 31, 2, pp. 221–226.

Chen, X.P., Zhu, Y.G., Hong, M.N., Kappler, A. & Xu, Y.X. (2008). Effects of different forms of nitrogen fertilizers on arsenic uptake by rice plants, *Environmental Toxicology and Chemistry*, 27, 4, pp. 881–887.

Clarridge, J.E. (2004). Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases, *Clinical Microbiology Reviews*, 17, 4, pp. 840–862.

Correira, P.R.M., Nomura, C.S. & Oliviera, P.M. (2003). Multielement determination of cadmium and lead in urine, by simultaneous electrothermalatomic absorption spectrometry with end-capped graphite tube, *Journal of Analytical Science*, 19, 11, pp. 1519–1523.

Flynn, A.N., Lyndon, C.A. & Church, D.L. (2013). Identification by 16S rRNA gene sequencing of an Actinomyces hongkongensis isolate recovered from a patient with pelvic actinomycosis, *Journal of Clinical Microbiology*, 51, 8, pp. 2721–2723.

Frankenberger, W.T.Jr. & Arshad, M. (2002). *Volatilisation of arsenic*. In: Frankenberger W.T.Jr. (ed.), Environmental chemistry of arsenic. Marcel Dekker, New York 2002.

Gremion, F., Chatzinotas, A. & Harms, H. (2003). Comparative 16S rDNA and 16S rRNA sequence analysis indicates that Actinobacteria might be a dominant part of the metabolically active bacteria in heavy metal-contaminated bulk and rhizosphere soil, *Environmental Microbiology*, 5, 10, pp. 896–907.

Groundwater Research Center (2010). *Performance report of prevention in environmental impact and environmental monitoring of gold mining project (Phu Thap Pha, Loei province)*, Faculty of Technology, Khon Kaen University, Khon Kaen, Thailand 2010.

Gupta, M. & Sarin, N.B. (2009). Heavy metal induced DNA changes in aquatic macrophytes: random amplified polymorphic DNA analysis and identification of sequence characterized amplified region marker, *Journal of Environmental Science*, 2, 5, pp. 686–690.

Henke, K.R. (2009). *Arsenic: Environmental Chemistry, Health Threats And Waste Treatment*, John Wiley & Sons, (http://onlinelibrary.wiley.com/book/10.1002/9780470741122 (29.06.2016)).

Hutchins, S.D., Davidson, M.S., Brierley, J.A. & Brierley, C.L. (1986). Microorganisms in reclamation of metals, *Annual Review of Microbiology*, 40, pp. 311–336.

Ignatiadis, I. & Battaglia-Brunet, F. (2005). *Applicability of anaerobic bacterial leaching as remediation technique for As-contaminated soils: batch, column and pilot experiments and economic assessment*. In: Uhlmann, O., Annokkee, G.J & Arendt, F. (eds.), Proceeding of the 9th International FZK/TNO Conference on Soil-Water Systems, Bordeau, October 2005.

Jackson, B.P., Seaman, J.C. & Bertsch, P.M. (2006). Fate of arsenic compounds in poultry litter upon land application, *Chemosphere*, 65, 11, pp. 2028–2034.

Kang, S.H., Evans, P., Morrison, M. & McSweeney, C. (2013). Identification of metabolically active proteobacterial and archaeal communities in the rumen by DNA- and RNA-derived 16S rRNA gene, *Journal of Applied Microbiology*, 115, 3, pp. 644–653.

Kozdrój, J. & Van Elsas, J.D. (2000). Bacterial community DNA extracted from soils polluted with heavy metals, *Polish Journal of Environmental Studies*, 9, 5, pp. 403–407.

Lindh, J.M., Terenius, O. & Faye, I. (2005). 16S rRNA gene-based identification of midgut bacteria from field-caught Anopheles gambiae sensu lato and A. funestus mosquitoes reveals new species related to known insect symbionts, *Applied Environmental Microbiology*, 71, 11, pp. 7217–7223.

Lyman, C.E., Newbury, D.E., Goldstein, J.I., Williams, D.B., Romig, A.D., Armstrong, J.T., Echlin, P., Fiori, C.E., Joy, D.C., Lifshin, E. & Klaus-Ruediger, P. (1990). *Scanning electron microscopy, X–ray microanalysis, and analytical electron microscopy: a laboratory workbook*, Plenum Press, New York 1990.

Neeratanaphan, L., Sudmoon, R. & Chaveerach, A. (2014a). Assessment of genotoxicity through ISSR marker in Pistia stratiotes induced by lead, *Environment Asia*, 7, 2, pp. 99–107.

Neeratanaphan, L., Sudmoon, R. & Chaveerach, A. (2014b). Genetic erosion in freshwater snail, Filopaludina martensi affected by lead and cadmium, *Applied Ecology and Environmental Research*, 12, 4, pp. 991–1001.

Neeratanaphan, L., Dechmon, S., Phonimdaeng, P., Khamon, P. & Intamart, S. (2015). Removal of lead from wastewater contaminated with chemical synthetic dye by Aspergillus terreus, *EnvironmentAsia*, 8, 2, pp. 45–55.

Page, M. & Page, C. (2002). Electroremediation of contaminated soils, *Journal of Environmental Engineering*, 128, 3, pp. 208–219.

Park, G.S., Kim, D.H., Lim, J.G. & Ohga, S. (2006). Heavy metal concentration and identification of microorganisms in soil under roadside trees of Daejeon city; Korea, *Journal of the Faculty of Agriculture, Kyushu University*, 51, pp. 53–56.

Shahedur, R., Ki-Hyun, K., Subbroto, K.S., Swaraz, A.M. & Dipak, K.P. (2014). Review of remediation techniques for arsenic (As) contamination: A novel approach utilizing bio-organisms, *Journal of Environmental Management*, 134, pp. 175–185.

Suresh, K., Prabagaran, S.R., Sengupta, S. & Shivaji, S. (2004). Bacillus indicus sp. nov., an As-resistant bacterium isolated from an aquifer in West Bengal India, *International Journal of Systematic and Evolutionary Microbiology*, 54, 4, pp. 1369–1375.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, *Molecular Biology and Evolution*, 28, 10, pp. 2731–2739.

Trung, T.T., Hetzer, A., Topfstedt, E., Gohler, A., Limmathurotsakul, D., Wuthiekanun, V., Peacock, S.J. & Steinmetz, L. (2011). Improved culture-based detection and quantification of Burkholderia pseudomallei from soil, *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 105, 6, pp. 346–351.

US EPA. (2002). *Supplemental guidance for developing soil screening levels for superfund sites*, Office of Solid Waste and Emergency Response, Washington DC 2002.

Valls, M. & Lorenzo, V.D. (2002). Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution, *FEMS Microbiology Reviews*, 26, 4, pp. 327–338.

Williams, P.N., Lei, M., Sun, G.X., Huang, Q., Lu, Y. & Deacon, C. (2009). Occurrence and partitioning of cadmium, arsenic and lead in mine impacted paddy rice: Hunan, *China, Environmental Science Technology*, 43, 3, pp. 637–642.

Zhou, L., Li, J., Lin, X. & Al-rasheid, K.A.S. (2011). Use of RAPD to detect DNA damage induced by nitrofurazone in marine ciliate, Euplotes vannus (Protozoa, Ciliphora), *Aquatic Toxicology*, 103, 4, pp. 225–232.