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Original Article

Isolation of arsenic-tolerant bacteria from arsenic-contaminated soil

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Abstract

The disposal of toxic heavy metals such as arsenic posed high risk to the environment. Arsenite [As(III)], a reduced form of arsenic, is more toxic and mobile than arsenate [As(V)]. The aim of this work was to isolate arsenic-tolerant bacteria from contaminated soil collected in Ronphibun District, Nakorn Srithammarat Province, followed by screening these bacteria for their ability to adsorb arsenite. Twenty-four bacterial isolates were obtained from samples cultivated in basal salts medium plus 0.1% yeast extract and up to 40 mM sodium-arsenite at 30°C under aerobic condition. From these, isolates B-2, B-3, B-4, B-21, B-25 and B-27 produced extracellular polymeric-like substances into the culture medium, which may potentially be used in the bioremediation of arsenic and other contaminants. All isolates displayed arsenite adsorbing activities in the ranges of 36.87-96.93% adsorption from initial concentration of 40 mM sodium-arsenite, without any arsenic transforming activity. Five isolates with the highest arsenite adsorbing capacity include B-4, B-7, B-8, B-10 and B-13 which adsorbed 80.90, 86.72, 87.08, 84.36 and 96.93% arsenite, respectively. Identification of their 16S rDNA sequences showed B-7, B-8, and B-10 to have 97%, 99% and 97% identities to *Microbacterium oxydans*, *Achromobacter* sp. and *Ochrobactrum anthropi*, respectively. Isolates B-4 and B-13, which did not show sequence similarity to any bacterial species, may be assigned based on their morphological and biochemical characteristics to the genus *Streptococcus* and *Xanthomonas*, respectively. Thus, both isolates B-4 and B-13 appear to be novel arsenite adsorbing bacteria within these genuses.

Keywords: arsenic, arsenic-tolerant bacteria, arsenite, bioadsorption

1. Introduction

Arsenic is a semi-metallic element, which may be found in a variety of form, viz. -3, 0, +3, +5 valencies. The most common arsenic species observed in the environment are the trivalent form arsenite [As(III)] and pentavalent form arsenate [As(V)]. Arsenate is often found co-precipitated with iron oxyhydroxide (FeOOH), which may be immobilized under acidic and moderately reducing conditions. Under reducing conditions, arsenic is found as arsenite which can co-precipitate with metal sulfides (Niggemyer *et al.*, 2001). In addition, numerous environmental factors also influence arsenic speciation in soil, such as pH, redox potential, the presence of other ions, organic matter content, soil texture, fungal or bacterial activities (Turpinen *et al.*, 1999).

The sources of environmental arsenic may derived 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, industry activities (Dowdle *et al.*, 1996; Stolz and Oremland, 1999; Liest *et al.*, 2000; Mandal and Suzuki, 2002).

The toxicity of different forms of arsenic decrease in the order: arsine>inorganic arsenite>organic arsenite> inorganic arsenate>organic arsenate>free arsenic (Mandal and Suzuki, 2002). Toxicity depends on factors such as physical state, gas, solution, or powder particle size, cell adsorption rate, elimination rate, and the nature of chemical substituents in the toxic compound (Anderson and Cook, 2004). The deleterious effects of arsenic to human health

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resulting from environmental contamination have been reported worldwide (Katsoyiannis *et al.*, 2002; Mandal and Suzuki, 2002). In Thailand, it was first reported from Ronphibun District in 1987, where local residents utilizing well-water were diagnosed with skin cancer. Soil and groundwater in the area were contaminated with up to 0.1% arsenopyrite and 100 times of the regulatory arsenic level of 0.01 mg/l in drinking water (Mandal and Suzuki, 2002).

Several remediation techniques for arsenic removal have been applied, e.g., ion exchange, adsorption with activated alumina and activated carbon, ultrafiltration, reverse osmosis, and complexation with metal ions followed by coagulation. These methods required large amount of chemical reagents, for example, adsorption and ion exchange are expensive when use in the event of high concentration of heavy metal ions. Furthermore, the treated sludge may be contaminated with treatment reagents resulting in secondary environmental pollution. Due to this apparent disadvantage of the above physico-chemical treatments, novel techniques for the reduction of contaminant toxicity in concerted with minimizing cost have been proposed.

Biological remediation techniques, either using living/dead cells or biosynthesized molecules have been examined (Katsoyiannis *et al.*, 2002). Studies have shown that both plant and microorganism are able to accumulate metal ions via processes such as transportation across the cell membrane, biosorption onto cell wall, entrapment in extracellular capsule, precipitation, oxidation-reduction reaction and biosorption to extracellular polysaccharide (Malik, 2004). Hyperaccumulating plant species, such as *Pityrogramma calomelanos* and *Pteris vitta*, were shown to accumulate arsenic in the form of arsenate at the leaf section (Visoottiviseth *et al.*, 2002). Studies have reported the ability of algae, fungi and bacteria to transform arsenite to arsenate and vice versa during their growth (Macy *et al.*, 2000; Hasegawa *et al.*, 2001; Visoottiviseth and Panviroj, 2001). *Desulfomicrobium* sp. BenR-B has been shown to reduce arsenate to arsenite via enzyme arsenate reductase (Macy *et al.*, 2000). The mechanisms involved in the microbial transformation and removal of arsenic from the environment included adsorption via reduction reaction by *Desulfomicrobium* sp. BenR-B, oxidation/reduction reaction by *Trichoderma harzianum* AS11 and *Trichosporon mucoides* SBUG801, and methylation reaction by *Paenibacillus* sp. and *Pseudomonas* sp. (Macy *et al.*, 2000; Hofmann *et al.*, 2001; Jenkis *et al.*, 2003)

In this report, we describes the isolation, effect of arsenic on bacterial growth and identification of arsenic-tolerant bacteria from contaminated soil in the area surrounding arsenic landfill site and from area within the Phytoremediation Project site (collaborative effort between Mahidol University and Ronphibun Subdistrict).

2. Materials and Methods

2.1 Soil collection

Soil samples were collected from Ronphibun District, Nakorn Srithammarat Province, where soil, sediment, and ground water in the area have been contaminated with arsenic for many years by decades of tin mining activities (Mandal and Suzuki, 2002). Sample collecting sites were within the area of the Phytoremediation Project and area surrounding arsenic landfill. Subsurface soil (from 0-15 cm in depth) were collected, placed in plastic bag and kept on ice or at 4ºC until further analysis.

The pH of soil sample was determined by shaking 10 g of soil in 20 ml of distilled water for 25 minutes followed by measurement with pH meter (model Delta 320, Mettler; UK).

2.2 Arsenic analysis by ICP-AES

One to two grams of soil samples were added to 10 ml 50% HNO_3, heated and refluxed at 95ºC 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 95ºC. This step was repeated one more time. The solution was evaporated until approximately 5 ml of the solution was left. After the solution cooled to room temperature, 2 ml deionized H_2O and 3 ml 35% H_2O_2 was added and heated to 95ºC. The solution was cooled to room temperature and added with 7 ml warmed 35% H_2O_2. The solution was again cooled to room temperature, added with 5 ml concentrated HCl and 10 ml deionized H_2O, and refluxed at 95ºC for 15 min. The digested solution was filtered to remove residual particulates and then diluted to 100 ml with deionized H_2O. Arsenic concentration was determined by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) (PerkinElmer, Inc.; USA).

2.3 Growth media for bacteria isolation

Two formulations of basal salt medium were used throughout this study. BSMY I used for the isolation of bacteria from arsenic contaminated soil samples contained (per liter): 1.0 g yeast extract, 0.3 g $(NH_4)_2SO_4$, 0.14 g $MgSO_4 \cdot 7H_2O$, 0.2 g $CaCl_2 \cdot 2H_2O$, 0.1 g NaCl, 0.05 g KH_2PO_4, 0.05 g K_2HPO_4, 0.6 mg H_3BO_3, 0.17 mg $CoCl_2 \cdot 6H_2O$, 0.09 mg $CuCl_2 \cdot 2H_2O$, 0.1 mg $MnCl_2 \cdot 4H_2O$, 0.22 mg $ZnCl_2$, 10 g glucose in 1 liter of Tris-HCl buffer (pH 8.0) (Yamamura *et al.*, 2003).

BSMY II used to study growth phase of bacteria in cultivation medium that added arsenic solution contained (per liter): 1.0 g yeast extract, 0.25 g NH_4Cl, 0.62 g $MgCl_2 \cdot 6H_2O$, 0.15 g $CaCl_2 \cdot 2H_2O$, 1.0 g NaCl, 0.14 g KH_2PO_4, 0.5 g KCl, 0.06 mg H_3BO_3, 0.12 mg $CoCl_2 \cdot 6H_2O$, 0.015 mg

$CuCl_2 \cdot 2H_2O$, 0.1 mg $MnCl_2 \cdot 4H_2O$, 0.22 mg $ZnCl_2$, 10 g glucose in 1 liter of deionized water adjusted to pH 6.8 with $NaHCO_3$ (modified from Kuai *et al.*, 2001).

Growth media were sterilized by autoclaving at 121°C for 15 minutes. Sodium-arsenite [Na-As(III)] was filter sterilized and added to the medium for the initial concentration of 5, 10, 20 and 40 mM.

2.4 Isolation and characterization of arsenic-tolerant bacteria

Two grams of each soil sample was dissolved in 20 ml 0.9% NaCl and shaken for 3 minutes. Then 5 millilitres of soil suspension was inoculated into 50 ml BSMY I containing 5 mM sodium-arsenite and incubated at room temperature on rotary shaker at 120 rpm for 3 days. Five milliliters of culture was transferred into fresh BSMY I medium containing 10 mM sodium-arsenite, and transferred twice into new medium that was supplemented with 20 and 40 mM sodium-arsenite. After growth was observed, 0.1 ml of culture was spreaded on BSMY agar that contained 40 mM sodium-arsenite and incubated at 30ºC for 3 days. Bacterial isolates that could tolerate the highest arsenite concentration were selected and identified by their morphological features and biochemical properties.

2.5 Effect of arsenic on bacterial growth

The selected bacterial strains were inoculated into BSMY II with 40 mM sodium-arsenite and then incubated at 30ºC, 150 rpm for 5 days. Samples were collected every 6-12 hours for the measurement of growth and arsenic concentration. Growth was determined via total protein by Lowry's method (A_{750}) and arsenic concentration (A_{880}) was determined spectrophotometrically. Determination of arsenic concentration in culture medium was performed with the method provided by Dhar *et al.* (2004). The effect of arsenic on growth was shown by extrapolating cell growth data and arsenic concentration data together.

2.6 Identification of bacteria isolates

1) Morphological characteristic

Bacteria isolates were grown in BSMY I with the addition of 40 mM sodium-arsenite and incubated at 30°C until either turbidity or colony was observed. Gram stain and cell morphology was investigated under microscope (1000x magnification).

2) Biochemical properties

Biochemical properties of the isolates were tested according to Bergey's Manual of Systematic Bacteriology (Krieg, 1984). The following properties were determined: motility, nitrite reduction, indole test, H_2S production, methyl red test, citrate utilization, starch hydrolysis, liquefaction, catalase test, oxidase test, oxidation/fermentation test (O/F), acid production from carbohydrates: glucose, lactose, sucrose.

3) 16S rDNA sequence analysis

Five isolates which showed the highest arsenic adsorption capacity was chosen for further identification by 16S rDNA sequence analysis. The genomic DNA was extracted from each isolate by boiling and freezing method (modified from Yamada *et al.*, 2002). Three milliliters of culture broth was centrifuged at 10,000 rpm for 5 min. Supernatant was decanted and cell pellet was washed with 300 µl of Tris-EDTA buffer (pH 7.0). Cell suspension was centrifuged at 10,000 rpm for 5 min and resuspended into 300 µl of Tris-EDTA buffer (pH 7.0). The suspension was placed in boiling water for 10 min and immediately cooled on ice-bath for 5 min. This step was repeated 3 times.

The 16S rDNA from the extracted DNA was amplified by PCR using 27F (5'- AGAGTTTGATCCTGGCTCAG -3') and 1492R (5'-ACGGCTACCTTGTTACGACTT-3') primers. The reaction mixtures composed of 5 µl 10xPCR buffer, 0.2 mM dNTPs, 1 µM each primer, 5µl DNA template, 2.5 units *Taq* DNA polymerase and sterile deionized water to a final volume of 50 ml. PCR profile was as follows: initial denaturation at 94°C for 3 min, followed by 25 cycles of denaturation (94°C, 1 min), annealing (50°C, 45 sec), extension (72°C, 2 min), and final extension at 72°C for 10 min. PCR product was purified by QIAquick PCR Purification Kit (QIAGEN, Inc.). DNA sequences were performed by Macrogen, Inc. (South Korea). The 16S rDNA gene sequences were BLAST searched against GenBank database (http://www.ncbi.nlm.nih.gov/) and phylogenetic analysis was performed using Treeview software (Page, 1996).

3. Results and Discussion

3.1 Arsenic and pH analyses of soil samples

A total of 25 soil samples were collected from areas within the Phytoremediation Project and area surrounding landfill containing arsenic contaminated soil. The pH and arsenic concentrations of collected soils were determined and found in the range of 5.39-8.19 and 42.14-1,010.96 ppm (or mg/kg soil), respectively (Table 1). Soils from the Phytoremediation Project had shown a more neutral pH (7.22-8.19) and slightly lower arsenic concentration (42.14-613.13 ppm) than soils from area surrounding arsenic-landfill, which had a slightly acidic pH of 5.39-7.20 and higher arsenic concentration (73.17-1,010.96 ppm). The arsenic levels shown here are relatively similar to those observed by Suwanmanee (1990), which reported arsenic concentration in the ranges of 50-5,300 mg/kg soil from samples obtained in the same vicinity. Arsenic concentration

Table 1. Arsenic concentration and pH value of the soil samples

Sample area	pH	Total arsenic concentration (mg As/kg soil)
Area within the Phytoremediation Project		
Site-1	7.35	123.52
Site-2	7.97	168.44
Site-3	7.88	102.15
Site-4	7.53	157.33
Site-5	7.71	140.07
Site-6	7.85	130.95
Site-7	7.22	42.14
Site-8	7.43	65.96
Site-9	8.00	94.76
Site-10	7.90	108.18
Site-11	7.95	140.77
Site-12	8.13	86.02
Site-13	7.51	613.13
Site-14	7.39	56.37
Area surrounding landfill containing arsenic contaminated soil		
Site-15	6.90	154.19
Site-16	6.95	73.17
Site-17	5.82	195.62
Site-18	7.02	91.49
Site-19	5.39	180.42
Site-20	5.58	102.00
Site-21	6.65	199.77
Site-22	6.72	132.43
Site-23	6.72	367.65
Site-24	6.26	1010.96
Site-25	7.20	641.94

and species are generally influenced by pH and redox potential of soil, soil texture, type of parent rock, organic matter and cation exchange capacity of soil (Mandal and Suzuki, 2002). Thus, arsenate is often the dominant species in aerobic environment or acidic soil and can absorb to organic matters. Sandy soil usually contains lower arsenic level as opposed to soil with high organic contents.

3.2 Isolation of arsenic-tolerant bacteria from contaminated soils

Indigenous bacteria from arsenic contaminated soils were firstly enriched in BSMY I broth with increased sodium-arsenite concentrations of 5, 10, 20 and 40 mM. The cultivated samples were then screened for arsenic-tolerant bacteria in BSMY I agar with the addition of 40 mM sodium-arsenite. In all, 45 bacterial strains were isolated from BSMY I medium at different sodium-arsenite concentrations, with 24 bacterial strains able to grow in the presence of 40 mM sodium-arsenite. This result suggested that the isolates may have developed metal resistance systems in an attempt to protect sensitive cellular components. It was observed that

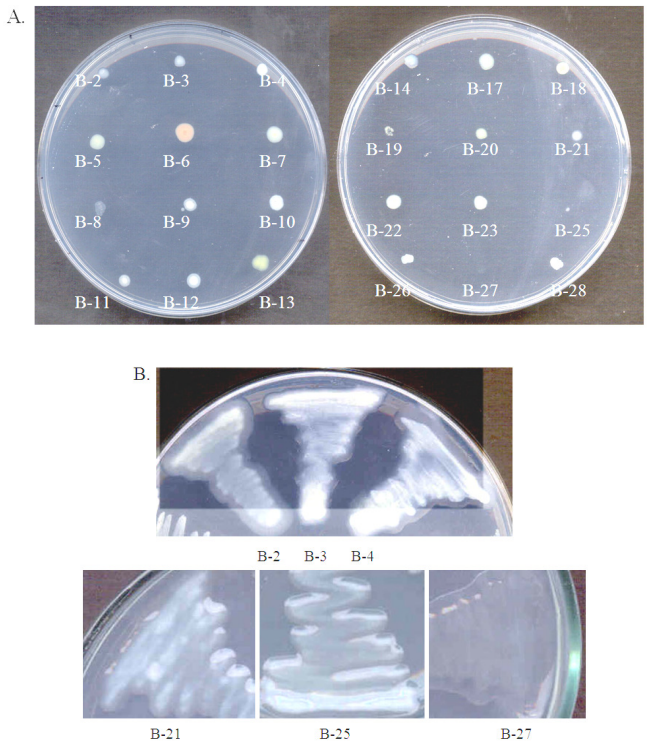

Figure 1. (A) Bacterial isolates grown on BSMY I agar supplemented with 40 mM sodium-arsenite at 30ºC for 3 days. (B) Isolates with extracellular polymeric-like substance surrounding the colonies when grown on BSMY I agar supplemented with 40 mM sodium-arsenite at 30ºC for 3 days.

most bacterial isolates had an off-white color, circular-shaped, translucent colony with flat plateau and smooth surface. Some isolates also had pale-orange (B-10 and B-19), pink (B-6 and B-18), yellow (B-9, B-13, B-20 and B-22) or colorless colonies (B-8 and B-11) (Figure 1A). Further observation indicated that five isolates (B-2, B-3, B-4, B-21, B-25 and B-27) produced extracellular polymeric-like substance, which may consisted of polysaccharide, protein or nucleic acid with functional group capable of binding to metal ion (Figure 1B). In general, microbial ability to grow at high metal concentration is found coupled with a variety of specific mechanisms of resistance and environmental factors. 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 *et al.*, 2002; Zoubilis *et al.*, 2004).

3.3 Effect of arsenic on bacterial growth

All 24 selected isolates showed increase lag and exponential phases of growth when grown in BSMY II with

the addition of 40 mM sodium-arsenite as compared to without arsenite addition (data not shown). This was probably due to the effect of arsenite which retarded bacterial growth. During cultivation, the level of arsenite in the growth medium of each isolate was decreased from its initial concentration while arsenate level was constant (data not shown), suggesting that arsenic was adsorbed to microbial cell rather than transformed from arsenite to arsenate. The optimal incubation time for arsenite adsorption by most isolates was shown to occur during exponential phase of growth, whereas arsenite adsorption by isolates B-9 and B-17 occurred during stationary phase of growth. Some studies had reported that microbial metal removal can be found in exponential, mid-exponential and stationary phases of growth (Wang *et al.*, 1997; Yilmaz, 2003). Moreover, it was found that isolates B-20, B-21, B-25, and B-27 showed little growth coupled with more than 45% arsenite removal. Isolates B-21, B-25, and B-27 were observed to produce extracellular polymeric-like substance which in these cases may play a role in arsenite removal in order to lower the toxic effect of the compound. Five isolates with the highest arsenite adsorption capacity were isolates B-4, B-7, B-8, B-10 and B-13, which decreased arsenite levels by 80.90%, 86.72%, 87.08%, 84.36% and 96.93%, respectively (Figure 2). Bacterial isolate with the lowest arsenite adsorption capacity was B-18 with 36.87% arsenite removed.

3.4 Identification of arsenic absorbing isolates

Morphological and biochemical characteristics of all 24 bacterial isolates were determined by Gram's staining and biochemical tests according to the Bergey's Manual of Systematic Bacteriology (Krieg, 1984). The investigation results indicated that sixteen isolates were Gram negative rod-shaped bacteria, two isolates were Gram negative coccoid-shaped bacteria and six isolates were Gram positive coccoid-shaped bacteria (Table 2). Gram negative bacteria have two layer of cell membrane enabling them to resist and

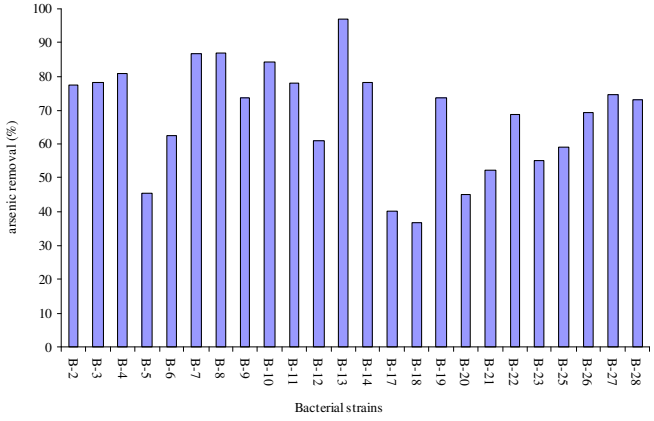

Figure 2. Arsenic removal efficiency of each bacterial isolate grown in BSMY II medium supplemented with 40 mM sodium arsenite at 30°C, 150 rpm for 5 days.

Table 2. Morphological and biochemical characteristics of isolated bacteria strains

Morphological and biochemical characteristic	Isolates																							
	B-2	B-3	B-4	B-5	B-6	B-7	B-8	B-9	B-10	B-11	B-12	B-13	B-14	B-17	B-18	B-19	B-20	B-21	B-22	B-23	B-25	B-26	B-27	B-28
Gram stain	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	-	-	-
Cell shape	rod	rod	cocci	cocci	rod	cocci	cocci	rod	rod	rod	rod	rod	rod	rod	cocci	cocci	cocci	rod	rod	cocci	rod	rod	rod	rod
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Indole test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methyl red test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Citrate utilization	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	+
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrite reduction	+	-	-	+	-	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+
H_2S production	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Catalase test	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	+	+	+
Oxidase test	+	+	+	-	-	-	+	+	+	+	+	-	+	-	-	-	-	+	-	-	+	+	-	+
Oxidation/Fermentation (O/F)	O	O	O	F	F	F	F	O	O	O	O	O	F	F	F	F	F	O	F	F	O	O	F	O
Gelatin liquefaction	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Acid production from carbohydrates:																								
Glucose	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	+	-	-	+	+	+	+
Lactose	+	-	-	+	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

grow at higher metal concentration than Gram positive bacteria. According to their observed morphological and biochemical characteristics, four isolates (B-6, B-17, B-22 and B-27) were designated to genus *Enterobacter*, three isolates (B-7, B-8 and B-21) to genus *Neisseria*, eight isolates (B-2, B-3, B-9, B-10, B-14, B-25, B-26 and B-28) to genus *Pseudomonas*, four isolates (B-5, B-18, B-20 and B-23) to genus *Staphylococcus*, two isolates (B-4 and B-19) to genus *Streptococcus*, two isolates (B-11 and B-12) to genus *Xanthobacter* and one isolate (B-13) to genus *Xanthomonas*.

Five isolates, i.e., B-4, B-7, B-8, B-10 and B-13, which displayed high arsenic adsorption capacity, were further identified by 16S rDNA analysis. The DNA extracts from each isolate were amplified with 27F-1492R primers and found to provide PCR products of 1,460-bp (data not shown). The PCR fragments were analyzed and the resulting 16S rDNA sequences indicated that the nearest phylogenetic relative of isolates B-7, B-8 and B-10 are *Microbacterium oxydans* (97% identity, 707/724), *Achromobacter* sp. (99% identity, 1378/1379) and *Ochrobactrum anthropi* (97% identity, 1106/1134), respectively (Figure 3). Although the above 16S rDNA analyses placed these isolates in a different genus than those identified by biochemical tests, it must be remember that DNA analysis provides us with the ability to clarify microorganism identity at species level opposed to group or genus level by biochemical tests. In resolving these differences in identity, the biochemical characteristics of *Microbacterium oxydans*, *Achromobacter* sp. and *Ochrobactrum anthropi* were reviewed and found to be as follows: *Microbacterium oxydans* is an aerobic bacterium which produces acid from sucrose, cannot utilized Simmons' citrate for growth, catalase-positive, oxidase and methyl red-negative (Schumann *et al.*, 1999). *Achromobacter* sp. is an obligated aerobic Gram-negative bacterium which produces acid from sucrose or hydrolyzed starch, reduces nitrate to nitrite, does not liquefy gelatin, does not produced H_2S and indole, and is oxidase-positive (Chester and Cooper, 1979). *Ochrobactrum anthropi* is an aerobic Gram-negative bacterium which produces acid from glucose, reduces nitrate to nitrite, and is oxidase- and catalase-positive (Laura *et al.*, 1996; Moller *et al.*, 1999). The above characteristics are identical to those observed from isolates B-7, B-8 and B-10 (Table 2). Thus, confirming the results obtained via 16S rDNA analyses.

In contrast, the 16S rDNA sequences of isolates B-4 and B-13 did not show similarity to any bacterial species suggesting that both may be novel arsenic absorbing strains. Based on their morphological and biochemical characteristics, isolates B-4 and B-13 were assigned to the genus *Streptococcus* and *Xanthomonas*, respectively (Figure 3).

The diversity of bacteria isolates is rather typical as

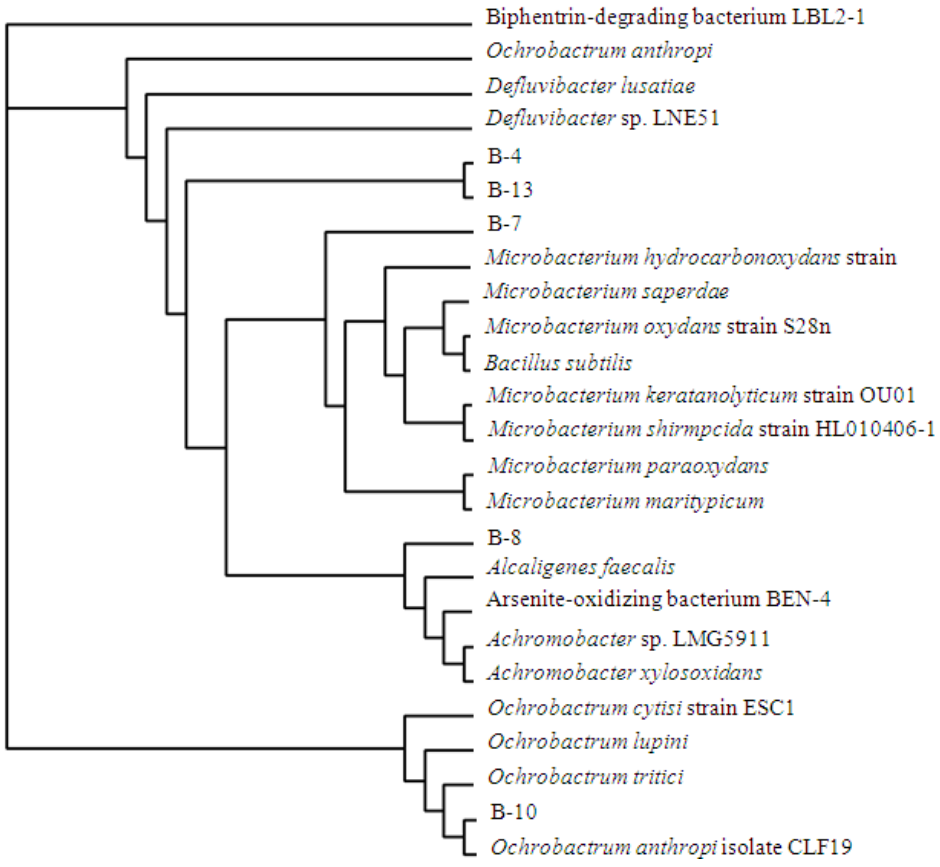

Figure 3. Phylogenetic analysis of arsenic-tolerant bacterial isolates B-4, B-7, B-8, B-10 and B-13.

difference of source and physico-chemical properties of arsenic contaminated samples have been shown to provide different bacteria isolates, such as *Acinetobacter*, *Aeromonas*, *Aureobacterium*, *Bacillus*, *Escherichia*, *Klebsiella*, *Micrococcus*, *Pseudomonas, Rhodococcus* and *Stenotrophomonas* (Clausen, 2000; Anderson and Cook, 2004).

4. Conclusion

It was found that all soil samples collected from the Phytoremediation Project and Arsenic landfill were contaminated by arsenic at a level of 15-fold higher than that found in terrestrial soil (1.5-3 ppm). From the soil samples, 45 bacterial isolates were able to grow in BSYM I with 5 mM sodium-arsenite and twenty-four isolates were able to grow with the addition of 40 mM sodium-arsenite. Sixteen isolates were aerobic, Gram-negative, and rod-shaped bacteria. Six isolates were aerobic, Gram-positive, and coccoid-shaped bacteria. Two isolates were aerobic, Gram-negative, coccoid-shaped bacteria. Six isolates, B-2, B-3, B-4, B-21, B-25 and B-27, produced extracellular polymeric-like substance onto the agar medium. This occurrence may results in the removal of arsenite by adsorption to the extracellular polymeric-like substance even at a low cellular biomass of isolates B-21, B-25 and B-27. In the case of isolates B-2, B-3 and B-4 the mechanisms of both cellular adsorption and extracellular polymeric-like substance adsorption may play a combined role in arsenite removal, as shown by the relatively high arsenite removal of more than 75% compared to only 45% by isolates B-21, B-25 and B-27.

Based on biochemical characteristics, all twenty-four isolates were assigned to either genus *Enterobacter*, *Neisseria*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Xanthobacter* or *Xanthomonas*. However, 16S rDNA sequence analysis of five isolates, B-4, B-7, B-8, B-10 and B-13, which showed the highest removal of arsenite from growth medium, suggested a different genus identity. The 16S rDNA sequence analyses of isolates B-7, B-8 and B-10 indicated that they belong to/or are closely related to *Microbacterium oxydans*, *Achromobacter* sp. and *Ochrobactrum anthropi*, respectively. While 16S rDNA sequence analyses of isolates B-4 and B-13 did not showed close sequence similarity to any bacterial species in the GenBank database. Therefore, based on their morphological and biochemical characteristics, isolates B-4 and B-13 may be novel arsenic absorbing strains belonging in the genus *Streptococcus* and *Xanthomonas*, respectively. Analysis of arsenite and arsenate levels during cultivation revealed that all isolates displayed arsenite adsorbing activities during either exponential or stationary phases of growth without the presence of any arsenic transforming activity. Bioadsorption of arsenite was found to be in the ranges of 36.87-96.93% from the initial concentration of 40 mM sodium-arsenite. The ability of the isolates which do not produce extracellular polymeric-like substance to remove large amount of arsenite from the cultivation medium are probably due solely to the cellular adsorption mechanism.

In order to fully appreciate the arsenic remediation potential of these five selected isolates, further studies on the use of viable or non-viable biomass as adsorbing material, optimizing the bioadsorption conditions, the possible recycling of this bioadsorbing material, and optimizing of the adsorbing and desorbing conditions need to be investigated.

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