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Isolation and Characterization of Arsenic Reducing Bacteria from Industrial Effluents and their Potential Use in Bioremediation of Wastewater

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Abstract.- The present study is aimed at assessing the ability of *Klebsiella oxytoca*, *Citrobacter freundii* and *Bacillus anthracis* to reduce arsenate into arsenite. *C. freundii* and *B. anthracis* could tolerate As (V) up to 290 mg/l. *K. oxytoca* resisted As up to 240 mg/l. *K. oxytoca* and *B. anthracis* showed optimum growth at pH 7 while *C. freundii* showed maximum growth at pH 5. *C. freundii* and *B. anthracis* showed optimum growth at 37ºC while the maximum growth of *K. oxytoca* was observed at 30ºC. *K. oxytoca* and *B. anthracis* were found sensitive against ampicillin while *C. freundii* showed resistance against it. *C. freundii* and *B. anthracis* were sensitive to erythromycin, kanamycine, nalidixic acid, and tetracycline while *K. oxytoca* was found resistant against these antibiotics. All bacterial strains were found to be sensitive to amoxicillin, chloramphenicol, neomycine, oxytetracycline, streptomycine, and polymixin B but all bacterial strains showed resistance against bacitracin. In arsC reductase crude assay *K. oxytoca*, *C. freundii* and *B. anthracis* showed high ability to reduce As(V) into As(III) 78%, 70%, and 84%, respectively. The bacterial isolates can be exploited for bioremediation of arsenic containing wastes, since they seem to have the potential to reduce the arsenate into arsenite form.

Key words: Arsenate reducing bacteria, *Klebsiella oxytoca*, *Citrobacter freundii*, *Bacillus anthracis*, bioremediation.

INTRODUCTION

Arsenic is a toxic metalloid naturally found as inorganic oxyanion arsenate As(V) and arsenite As(III) species. Presently, arsenic contamination of drinking water constitutes an important public health problem in numerous countries throughout the world (Smith *et al*., 2002). The World Health Organization recommends a provisional drinking water guideline of 10 ppb. Arsenic is a known human carcinogen (Hughes, 2002; Shi *et al*., 2004). Arsenic toxicity causes skin lesions, rhagades, and damage mucous membranes, digestive, respiratory, circulatory and nervous system and more over it is associated with skin, liver and lung cancers (Wang *et al.,* 2001).

Arsenite has the ability to bind to sulfhydryl groups of proteins and dithiols such as glutaredoxin. On the other hand, arsenate is a chemical analog of phosphate and can inhibit oxidative phosphorylation (Ordonez *et al.,* 2005). It may interfere with the DNA repair system or DNA methylation state, inhibition of p53 and telomerase activities (Chou *et al*., 2001; Wang *et al.,* 2001), oxidative stress, promotion of cell proliferation and signal transduction pathways leading to the activation of transcription factors (Wu *et al*., 1999). It has also been shown that arsenic induces DNA damage via the production of reactive oxygen species (Matsui *et al.,* 1999).

Conventional methods for removing metals from industrial effluents include chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies and evaporation recovery (Ahluwalia and Goyal, 2007). These processes may be ineffective or extremely expensive especially when the metals in solution are in the range of 1-100 mg/l (Nourbakhsh *et al.,* 1994). Therefore, it is important to develop an innovative, low cost and eco-friendly method for removal of toxic heavy metal ions from the wastewater.

A wide variety of microorganisms is capable of growth in the presence of heavy metal ions and

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tolerates high concentrations (Nies, 1992; Gaballa and Helmann, 2003; Rehman *et al.,* 2007). Anderson and Cook (2004) have reported strains of *Aeromonas*, *Exiguobacterium*, *Acinetobacter*, *Bacillus* and *Pseudomonas*, that can tolerate high concentrations of arsenic species (upto 100 mM arsenate or upto 20 mM arsenite). Since heavy metals are ubiquitously present in our environment, microorganisms have developed mechanisms to resist the toxic effects of these heavy metals (White and Gadd, 1986). Several bacteria (Cervantes *et al*., 1994; Oremland *et al.,* 2001) belonging to the genera *Acidithiobacillus*, *Bacillus*, *Deinococcus, Desulfitobacterium* and *Pseudomonas* (de Vicente *et al*., 1990; Dopson *et al.,* 2001; Niggemyer *et al*., 2001; Suresh *et al.,* 2004) have been reported to be resistant to arsenic.

The present study deals with the isolation and characterization of arsenic resistant bacteria from a contaminated environment, the ability of the bacteria to reduce arsenate, and optimization of temperature and pH for maximum arsenate reduction.

MATERIALS AND METHODS

Sample collection

Wastewater samples were collected in screw capped sterilized bottles from Sheikhupura (Pakistan). Some physicochemical parameters of wastewater *viz.*, temperature, pH, dissolved oxygen and arsenic (µg/ml) were measured (APHA, 1989).

Isolation of As resistant bacteria

For isolation of arsenic resistant bacteria, 100 µl of the wastewater sample was spread on Luria-Bertani (LB) agar plates containing 100 µg of As(III)/ml of the medium. LB agar plates were prepared by dissolving 1 g NaCl, 1 g tryptone and 0.5 g yeast extract in 100 ml distilled water, pH adjusted at 7 to 7.2 and then 1.5 g agar was added in the 250 ml flasks. The medium was autoclaved at 121ºC for 15 minutes. The growth of the bacterial colonies was observed after 24 hours of incubation at 37ºC. Effect of As(III) on the growth of bacterial isolates was determined in acetate minimal medium which contained (g/l): NH_4Cl, 1.0; $CaCl_2.H_2O$, 0.001; $MgSO_4$.7H2O, 0.2; $FeSO_4$ .7H_2O, 0.001; sodium acetate, 5; yeast extract, 0.5; K2HPO_4, 0.5 (pH 7) supplemented with $Na_2HAsO_4.7H_2O$ (Pattanapipitpaisal *et al*., 2001). It was again incubated at 37 ºC for 24 hours. This process was repeated with successively higher concentrations of As (III) until the minimum inhibitory concentration (MIC) of the bacterial isolate was obtained. Experiments were carried out in duplicate.

Identification of the bacterial isolates

For biochemical characterization the isolates were tested for catalase activity, motility, oxidase acivity, nitrate reduction, and hydrolysis of casein according to Benson (1994). Some specific tests were also performed for further characterization of the isolates up to species level such as blood agar test, MacConkey agar test, utilization of different sugars, Voges-Proskauer test, and hydrolysis of starch. The procedures of these biochemical tests were taken from Cappuccino and Sherman (2001).

For molecular identification, genomic DNA was extracted as described by Carozzi *et al. (*1991) and the 16S rRNA gene was amplified by PCR using 16S rRNA primers (RS-1; 5′-AAACTC-AAATGAATTGACGG-3′, and RS-3; 5′-ACGGGCGGTGTGTAC-3′) (Rehman *et al*., 2007). PCR was performed by initial denaturation at 94°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, extension at 72°C for 2 minutes and a final extension at 72°C for 5 minutes. The PCR product of 0.5kb was removed from the gel and cloned in pTZ57R/T vector. The amplified 16S rRNA gene was purified with a Fermentas purification kit (#K0513) and the amplified products were electrophoresed on 1% agarose gel. Sequencing was carried out by Genetic analysis system model CEQ-800 (Beckman) Coulter Inc. Fullerton, CA, USA. The 16S rRNA gene sequences were compared with known sequences in the GenBank database to identify the most similar sequence alignment.

Determination of optimum growth conditions

For optimum growth of the bacterial isolates, two parameters *i.e*., temperature and pH were considered. For determination of optimum temperature, 5 ml LB broth was added in 4 sets,

each of three test tubes, autoclaved and inoculated with 20 µl of freshly prepared culture of each bacterial isolate by overnight growth at 37ºC in LB broth. The four sets of tubes were incubated at 25ºC, 30ºC, 37ºC and 42ºC. After an incubation period of 12 h, their absorbance was taken at 600 nm using a LAMBDA 650 UV/Vis Spectrophotometer (PerkinElmer, USA). For determination of optimum pH, test tubes having 5 ml LB broth were prepared in 5 sets, each containing 3 test tubes and their pH was adjusted at 5.0, 6.0, 7.0, 8.0, and 9.0 then autoclaved. These tubes were inoculated with 20 µl freshly prepared culture of each bacterial isolate. After an incubation period of 12 h, their absorbance was taken at 600 nm.

Effect of As on bacterial growth

Growth curves of bacterial isolates were determined in acetate minimal medium containing arsenite (100 µg/ml). For each bacterial isolate 50 ml medium was taken in one set consisting of 3 flasks, autoclaved and then inoculated with 50 µl of the freshly prepared inoculums. The cultures were incubated at their respective temperature in an incubator shaker at 150 rpm. An aliquot of culture was taken at regular intervals (0, 4, 8, 12, 16, 20, 24, 28, and 32 hours) to measure absorbance at 600 nm.

Determination of antibiotic resistance

Thirteen different antibiotic discs were used to check the resistance or sensitivity of locally isolated As-resistant bacterial isolates. For this purpose antibiotic discs were placed on agar plates with bacterial cultures. The plates were incubated at 37^{o}C for *C. freundii* and *B. anthracis* and at 30oC for *K. oxytoca* for overnight. After 15 hours of incubation diameter of the clear zone around the antibiotic discs was measured with the help of scale in millimeters and results were recorded in terms of sensitive (S) or resistant (R).

Reduction of arsenate by bacteria

In order to determine the ability of bacterial isolates to reduce As (V) to As (III), the NADPH oxidation method was used (Anderson and Cook, 2004). Cells were grown to log phase (overnight grown culture) in 250 ml of acetate minimal medium supplemented with 100 µg/ml of arsenate, spun down at 14000 (6500 x g) for 5 min and pellet was washed twice in 50 ml of reaction buffer (10mM Tris, pH 7.5, with 1mM Na_2EDTA and 1 mM $mgCl_2$), and finally resuspended in 15 ml of reaction buffer. Cells were lysed by sonication, and centrifuged at 14000 (6500 x g) for 5 min and supernatant was used for arsC enzyme assay. The NADPH oxidation was initiated at 37^{o}C by mixing 50 µl of crude extract in 820 µl of reaction buffer, 30 µl of 10mm DTT, 50 µl of 2 mM arsenate, and 50 µl of 3 mM NADPH (final concentratuion 0.15mM). Arsenate concentration of 100 µg/ml and control (no arsenic) were assayed. Measurements were taken at 340 nm, where 0.15 mM NADPH has an absorbance of approximately 1.0. Absorbance decreases as NADPH is oxidized coupled to arsenate reduction to arsenite. The percentage reduction of arsenate with reference to NADPH oxidation was calculated.

Statistical analysis

Observations were made and all the experiments run in triplicate. At least three separate flasks were usually maintained for one treatment. Each time three readings were taken, their mean, and standard error of the mean were calculated.

RESULTS

Physicochemical characteristics of wastewater

Some physicochemical characteristics of industrial wastewater were ascertained, from where arsenic tolerant bacteria were isolated. The temperature of different samples ranged between 28ºC to 34ºC, pH ranged between 6.0 and 7.8, and As ranging between 1.1 0±0.04 and 1.90 ±0.03 µg/ml.

Identification of the bacterial isolates

Biochemical characteristics of the As-resistant bacterial isolates are given in Table I. The partially amplified (500bp) and sequenced 16S rRNA gene from local isolates (SB1, SB2 and SA1) was uploaded to the NCBI (National Center for Biotechnology Information) website to search for similarity to known DNA sequences and to confirm the species of this local isolate. The nucleotide

sequences coding for the 16S rRNA gene after BLAST query revealed that this gene is 86% homologous to *Klebsiella oxytoca* (SB1), 94% homologous to *Citrobacter freundii* (SB2) and 96% homologous to *Bacillus anthracis* (SA1). The nucleotide sequences coding for the 16S rRNA gene of *K. oxytoca*, *C. freundii* and *B. anthracis* have been submitted to the GenBank database under accession numbers.

Table I.- Biochemical characteristics of the As-resistant bacterial isolates.

Biochemical tests	*K. oxytoca*	*C. freundii*	*B. anthracis*
Gram staining	-ve rods	-ve rods	+ve rods
Catalase test	+ve	+ve	-ve
Urease test	+ve	-ve	-ve
Gelatin hydrolysis test	-ve	+ve	+ve
Motility test	-ve	-ve	-ve
Glucose fermentation test	-ve	+ve	+ve
Fructose fermentation test	-ve	+ve	+ve
Lactose fermentation test	-ve	+ve	-ve
MRVP test	+ve	+ve	-ve
Citrate test	+ve	+ve	-ve
H_2S production test	-ve	+ve	+ve
Blood agar test	+ve	+ve	+ve
MacConkey agar test	+ve	+ve	-ve
Oxidase test	+ve	+ve	+ve
Indole test	-ve	-ve	-ve
Casein hydrolysis test	-ve	-ve	-ve
Nitrate reduction test	+ve	+ve	+ve

+ve = Positive; -ve = Negative

Optimum pH and temperature

The most suitable temperature for growth of *C. freundii* and *B. anthracis* was found to be 37°C while the optimum temperature for the growth of *K. oxytoca* was 30ºC (Fig. 1). *K. oxytoca* and *B. anthracis* showed optimum growth at pH 7 while *C. freundii* showed maximum growth at pH 5 (Fig. 2).

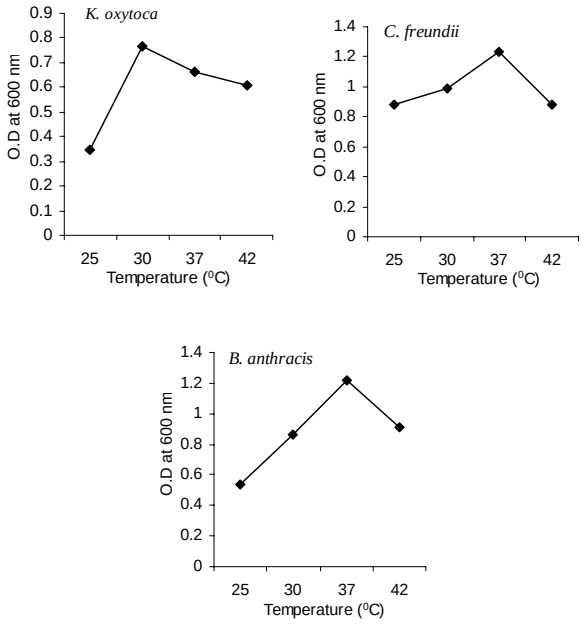

Fig. 1. Effect of temperature on the growth of bacterial isolates growing in LB medium.

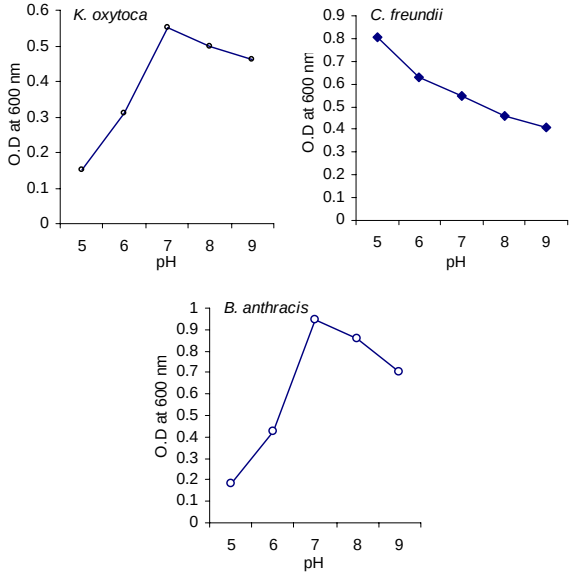

Fig. 2. Effect of pH on the growth of bacterial isolates growing in LB medium.

Growth curves

The growth curve pattern was studied by growing the organism in the presence of arsenite

(100 µg/ml) and comparing with the control culture in which no metal ions were added. Although the growth pattern of *K. oxytoca, C. freundii* and *B. anthracis* was not significantly different from those of control but the growth rate of bacterial isolates was lower in the presence of As(III. The lag phas is slightly delayed in the presence of As(III) in all bacterial isolates but in *C. freundii* it was extended up to 12 hours. The growth pattern is shown in Figure 3.

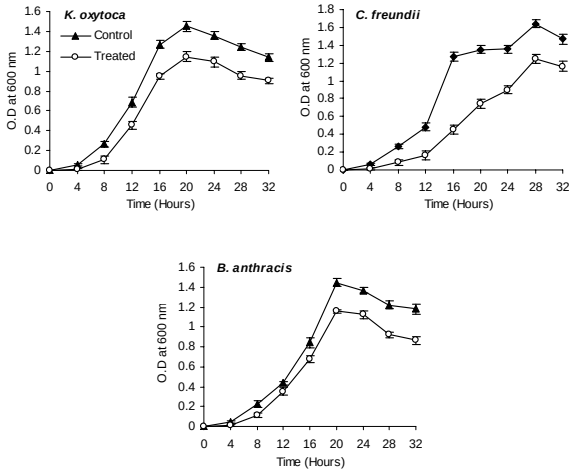

Fig. 3. Effect of arsenite concentration (100 µg/ml) on the cell growth of *K.oxytoca, C.freundii,* and *B.anthracis* in acetate minimal medium after incubation at their respective temperatures.

Bacterial antibiotic resistance

K. oxytoca and *B. anthracis* were found sensitive against ampicillin while *C. freundii* showed resistance against it. *C. freundii* and *B. anthracis* were sensitive to erythromycin, kanamycine, nalidixic acid, and tetracycline while *K. oxytoca* was found resistant against all these antibiotics. *K. oxytoca* was sensitive to vancomycine while other two bacterial isolates, *C. freundii* and *B. anthracis,* were found resistant against it. All three isolates were sensitive to amoxicillin, chloramphenicol, neomycine, oxytetracycline, streptomycine, and polymixin B. All bacterial strains have shown resistance against bacitracin (Table II).

Arsenate reduction ability of the bacterial isolates

In arsC reductase crude assay the bacterial strains, *K. oxytoca*, *C. freundii* and *B. anthracis* showed their ability to reduce As(V) into As(III) 78%, 70%, and 84%, respectively. These results indicate that bacteria can influence the arsenic speciation in the environment.

Table II.- Resistance of antibiotics by As-resistant bacterial strains.

Antibiotic	*K. oxytoca*	*C. freundii*	*B. anthracis*
Ampicillin	S (0.08mm)	R	S (0.08mm)
Amoxicilin	S (0.12mm)	S (0.08mm)	S (0.09mm)
Bacitracin	R	R	R
Chloramphenicol	S (0.15mm)	S (0.12mm)	S (0.13mm)
Eryhromycin	R	S (0.02mm)	S (0.02mm)
Kanamycine	R	S (0.09mm)	S (0.09mm)
Neomycine	S (0.11mm)	S (0.10mm)	S (0.10mm)
Nalidaixic acid	R	S (0.06mm)	S (0.07mm)
Oxytetracycline	S (0.06mm)	S (0.11mm)	S (0.09mm)
Streptomycine	S (0.11mm)	S (0.09mm)	S (0.08mm)
Tetracycline	R	S (0.06mm)	S (0.11mm)
Vancomycin	S (0.06mm)	R	R
Polymixin B	S (0.08mm)	S (0.07mm)	S (0.06mm)

S: Sensitive; R: Resistant

DISCUSSION

Microorganisms are known to play an important role in the biochemical cycle of arsenic, through its conversion to species with different solubility, mobility, bioavailability and toxicity (Silver and Phung, 2005). A variety of mechanisms exists for the removal of heavy metals from aqueous solution by bacteria, fungi, ciliates, algae, mosses, macrophytes and higher plants (Holan and Volesky, 1994; Pattanapipitpaisal *et al*., 2002; Rehman *et al*., 2007). The cellular response to the presence of

metals includes various processes such as biosorption by cell biomass, active cell transport, binding by cytosolic molecules, entrapment into cellular capsules, precipitation and oxidation-reduction reactions (Gadd, 1990; Lovely and Coates, 1997) as well as protein-DNA adduct formation (Zhitkovitch and Costa, 1992) and induction of stress proteins (Ballatori, 1994).

The arsenic-resistant bacteria isolated in this study were, *K. oxytoca*, *C. freundii* and *B. anthracis*, based on phylogenetic analysis of 16S rDNA sequence. Arsenite resistant bacteria have also been isolated from industrial effluents by several researchers (de Vincente *et al*., 1990; Anderson and Cook, 2004; Escalante *et al*., 2009). During the present investigation two bacterial strains, *C. freundii* and *B. anthracis,* could tolerate As (V) up to 290 mg/l while *K. oxytoca* was able to resist As up to 240 mg/l.

Berg *et al.* (2005) described elevated antibiotic resistance in copper-amended field using culture-based assays. Copper resistant isolates had a higher incidence of antibiotic resistance as compared to copper sensitive isolates, indicating that these metal and antibiotic traits are associated. Present study also supports the hypothesis that metal exposure results in increased frequency of antibiotic tolerance in bacteria. In the present study *C. freundii* and *B. anthracis* were sensitive to erythromycin, kanamycine, nalidixic acid, and tetracycline while *K. oxytoca* was found to be resistant to these antibiotics. *K. oxytoca* showed sensitivity against vancomycine while *C. freundii* and *B. anthracis* were found to resist it. All three isolates were sensitive to amoxicillin, chloramphenicol, neomycine, oxytetracycline, streptomycine, and polymixin B. All bacterial strains have shown resistance against bacitracin (Table II).

One potential method is microbially catalyzed reduction of As(V) to As(III), which is reported by many workers (Mukhopadhyay *et al.,* 2002; Anderson and Cook (2004; Silver and Phung, 2005; Escalante *et al*., 2009). Ars cytoplasmic arsenate reductase is found widely in microbes, and the arsC gene occurs in ars opetrons in most bacteria with total genomes measuring 2 Mb or larger as well as in some archaeal genomes (Silver and Phung, 2005). There are three unrelated clades of ArsC sequences which share a common biochemical function but have no evolutionary relationship (Mukhopadhyay *et al.,* 2002). These are (i) a glutaredoxin-glutthione-coupled enzyme, like that found associated with both the arsenite oxidase of *Alcaligenes* and the respiratory arsenate reductase of *Shewanella*, as well as many plasmids and chromosomes of gram-negative bacteria; (ii0 a less-well defined glutaredoxin-dependent arsenate reductase found in yeast; (iii) a group of thioredoxin-coupled arsenate reductases found both in gram-positive and negative proteo (Martin *et al.,* 2001). The *Pseudomonas aeruginosa* genome has separate genes for glutaredoxin and thioredoxin-coupled Ars reductases (Li *et al*., 2003).

Heavy metals in the environment select and maintain microbes possessing genetic determinants which confer resistance to the toxic compounds. In addition to chromosomal genes that function for uptake of inorganic arsenic as alternative substrates to useful nutrients, many microbes possess genes that specifically confer resistance to inorganic arsenic, both arsenate (As(V))and arsenite (As(III)), as their natural primary substrates (Silver and Phung, 1996; Rosen, 1999). In bacteria, these resistance determinants are often found on plasmid, which has facilitated their study at the molecular level (Silver and Phung, 2005). During the present investigation arsC reductase of *K. oxytoca*, *C. freundii* and *B. anthracis* showed its ability to reduce As(V) into As(III) 78%, 70%, and 84%, respectively. Cervantes *et al.* (1994) described that arsenate reduction in bacteria is catalyzed via the ars operon encoding an arsenate reductase (*arsC*) and an arsenite efflux pump (*arsB*). (It seems illogical to convert a less toxic compound to a more toxic form, but ArsC activity is closely coupled with efflux from the cells (Gatti *et al.,* 2000) so that intracellular arsenite never accumulates. Arsenate reductases from plasmids pl258 (Silver and Phung, 1996) and R773 (Aposhian, 1997) both reduce arsenate and both confer arsenate resistance.

CONCLUSION

In the present study *C. freundii* and *B. anthracis* could tolerate As (V) up to 290 mg/l while

K. oxytoca was able to resist As up to 240 mg/l. The bacterial strains showed also high level of arsenic reduction potential and could therefore represent good candidates for wastewater bioremediation processes. Further study on these bacterial strains is needed to understand the mechanism of high resistance and ability to reduce As(V) into AS(III).

REFERENCES

AHLUWALIA, S. S. AND GOYAL, D., 2007. Microbial and plant derived biomass for removal of heavy metals from wastewater. *Bioresour. Technol*., **98:** 2243-2257.

ANDERSON, C. R. AND COOK, G. M., 2004. Isolation and characterization of arsenate-reducing bacteria from arsenic contaminated sites in New Zealand. *Curr. Microbiol*., **48:** 341-347.

APOSHIAN, H. V., 1997. Enzymatic methylation of arsenic species and other new approaches to arsenic toxicity. *Annu. Rev. Pharmacol. Toxicol*., **37:** 397-419.

APHA, 1989. Standard methods for the examination of water and wastewater. APHA, 18th ed. Washington, DC.

BALLATORI, N., 1994. Glutathione mercaptides as transport forms of metals. *Adv. Pharmacol.*, **27:** 271-298.

BERG, J., TOM-PETERSEN, A. AND NYBROE, O., 2005. Copper amendment of agricultural soil selects for bacterial antibiotic resistance in the field. *Lebl. Appl. Microbiol.,* **40**: 146-151.

CERVANTES, C., Ji, G., RAMIREZ, J. L. AND SILVER, S., 1994. Resistance to arsenic compounds in microorganisms. *FEMS Microbiol. Rev*., **15:** 355–367.

CHOU, W. C., HAWKINS, A. L., BARRETT, J. F., GRIFFIN, C. A. AND DANG, C. V., 2001. Arsenic inhibition of telomerase transcription leads to genetic instability. *J. Clin. Invest*., **108:** 1541–1547.

CAROZZI, N.B., KRAMER, V.C., WARREN, G.W., EVOLA, S. AND KOZIEL, M.G., 1991. Prediction of insecticidal activity of *Bacillus thuringiensis* strains by polymerase chain reaction product profiles. *Appl. environ. Microbiol*., **57:** 3057-306.

DE-VICENTE, A., AVILES, M., CODINA, J. C., BORREGO, J. J. AND ROMERO, P., 1990. Resistance to antibiotics and heavy metals of *Pseudomonas aeruginosa* isolated from natural waters. *J. Appl. Bacteriol*., **68:** 625–632.

DOPSON, M., LINDSTROM, E. B. AND HALLBERG, K. B., 2001. Chromosomally encoded arsenical resistance of the moderately thermophilic acidophile *Acidithiobacillus caldus*. *Extremophiles*, **5:** 247–255.

ESCALANTE, G., CAMPOS, V. L., VALENZUELA, C., YANEZ, J., ZAROR, C. AND MONDACA, M.A., 2009. Arsenic resistant bacteria isolated from arsenic contaminated river in the Atacama desert (Chile). *Bull. environ. Contam. Toxicol*., **83:** 657-661.

GABALLA, A. AND HELMANN, J. D., 2003. *Bacillus subtilis* CPx-type ATPases: characterization of Cd, Zn, Co and Cu efflux systems. *Biometals*, **16:** 497-505.

GADD, G. M., 1990. Heavy metal accumulation by bacteria and other microorganisms. *Experientia,* **46:** 834-840.

GATTI, D. L., MITRA, B. AND ROSEN, B. P., 2000. Bacterial soft metal ATPases. *J. Biol. Chem.*, **275:** 34009-34012.

HOLAN, Z. R. AND VOLESKY, B., 1995. Accumulation of Cd, lead and nickel by fungal and wood biosorbents. *Appl. Biochem. Biotechnol*., **53:** 133-146.

HUGHES, M. F., 2002. Arsenic toxicity and potential mechanisms of action. *Toxicol. Lett*., **133:** 1–16

LI, R., HAILE, J.D. AND KENNELLY, P.J., 2003. An arsenate reductase from *Synechocystis* sp. strain PCC 6803 exhibits a novel combination of catalytic characteristics. *J. Bact*., **185:** 6780-6789.

LOVELY, D. R. AND COATES, J. D., 1997. Bioremediation of metal contamination. *Curr. Opin. Biotechnol*., **8:** 285-289.

MARTIN, P., DEMEL, S., SHI, J., ROSEN, B. P. AND EDWARDS, B. F. P., 2001. Insights into the structure, salvation, and mechanism of ArsC arsenate reductase, a novel arsenic detoxification enzyme. *Structure*, **9:** 1071-1081.

MATSUI, M., NISHIGORI, C., TOYOKUNI, S., TAKADA, J., AKABOSHI, M., ISHIKAWA, M., IMAMURA, S. AND MIYACHI, Y., 1999. The role of oxidative DNA damage in human arsenic carcinogenesis: detection of 8-hydroxy-2′-deoxyguanosine in arsenic-related Bowen's disease. *J. Invest. Dermatol*., **113:** 26-31.

MUKHOPADHYAY, R., ROSEN, B. P., PHUNG, L. T. AND SILVER, S., 2002. Microbial arsenic: from geocycles to genes. *FEMS Microbiol. Rev*., **26:** 311-325.

NIES, D. H., 1992. Resistance to cadmium, cobalt, zinc and nickel in microbes. *Plasmid*, **27:** 17-28.

NIGGEMYER, A., SPRING, S., STACKEBRANDT, E. AND ROSENZWEIG, R. F., 2001. Isolation and characterization of a novel As(V)-reducing bacterium: implications for arsenic mobilization and the genus *Desulfitobacterium*. *Appl. environ. Microbiol*., **67:**5568–5580.

NOURBAKHSH, M., SAG, Y., OZER, D., AKSU, Z., KUTSAL, T. AND CALGAR, A., 1994. A comparative study of various biosorbents for removal of chromium (VI) ions from industrial wastewater. *Process Biochem*., **29:** 1-5.

ORDONEZ, E., VALBUENA, N., GIL, J.A. AND MATEOS, L.M., 2005. Analysis of genes involved in arsenic resistance in *Corynebacterium glutamicum* ATCC 13032. *Appl. environ. Microbiol.,* **71:** 6206-6215.

OREMLAND, R. S., NEWMAN, D. K., KAIL, B. W. AND STOLZ, J. F., 2001. Bacterial respiration of arsenate and its significance in the environment. In: *Environmental chemistry of arsenic* (ed. W. T. Frankenberger Jr), pp. 273–296. Marcel Dekker, New

York.

PATTANAPIPITPAISAL, P., MABBETT, A. N., FINLAY, J. A., BESWICK, A. J., PATERSON-BEEDLE, M., ESSA, A., WRIGHT, J., TOLLEY, M. R., BADAR, U., AHMED, N., HOBMAN, J. L., BROWN, N. L. AND MACASKIE, L. E., 2002. Reduction of Cr (VI) and bioaccumulation of chromium by Gram-positive and Gram-negative microorganisms not previously exposed to Cr-stress. *Environ. Technol*., **23:** 731-745.

PATTANAPIPITPAISAL, P., BROWN, N. L. AND MACASKIE, L. E., 2001. Chromate reduction and 16S rRNA identification of bacteria isolated from a Cr (VI)-contaminated site. *Appl. Microbiol. Biotechnol.*, **57:** 257-261.

REHMAN, A., ALI, A., MUNEER, B. AND SHAKOORI, A.R., 2007. Resistance and biosorption of mercury by bacteria isolated from industrial effluents. *Pakistan J. Zool*., **39:** 137-146.

ROSEN, B. P., 1999. Families of arsenic transporters. *Trends Microbiol*., **7:** 207-212.

SILVER, S. AND PHUNG, L. T., 1996. Bacterial heavy metal resistance: new surprises. *Ann. Rev. Microbiol*., **50:** 753–789.

SILVER, S. AND PHUNG, L. T., 2005. Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. *Appl. environ. Microbiol*., **71:** 599–608.

SMITH, A.H., LOPIPERO, P.A., BATES, M.N. AND STEINMAUS, C.M., 2002. Public health. arsenic epidemiology and drinking water standards. *Science*, **296:** 2145–2146.

SHI, H., SHI, X. AND LIU, K-J., 2004. Oxidative mechanisms of arsenic toxicity and carcinogenesis. *Mol. Cell. Biochem*., **255:** 67–78.

SURESH, K., REDDY, G. S. N., SENGUPTA, S. AND SHIVAJI, S., 2004. *Deinococcus indicus* sp. nov., an arsenic-resistant bacterium from an aquifer in West Bengal, India. *Int. J. Syst. Evol. Microbiol.,* **54:** 457–461.

WANG, T. S., HSU, T. Y., CHUNG, C. H., WANG, A. S., BAU, D. T. AND JAN, K. Y., 2001. Arsenite induces oxidative DNA adducts and DNA–protein cross-links in mammalian cells. *Free Radical Biol. Med*., **31:** 321–330.

WHITE, C. AND GADD, G., 1986. Uptake and cellular distribution of copper, cobalt and cadmium in strains of *Saccharomyces cerevisiae* cultured on elevated concentration of these metals. *FEMS Microbiol. Rev*., **38:** 227-283.

WU, W., GRAVES, L. M., JASPERS, I., DEVLIN, R. B., REED, W. AND SAMET, J. M., 1999. Activation of the EGF receptor signaling pathway in human airway epithelial cells exposed to metals. *Am. J. Physiol*., **277:** 924–931.

ZHITKOVITCH, A. AND COSTA, M., 1992. A simple sensitivity assay to detect DNA-protein crosslinks in intact cells and *in vivo*. *Carcinogenesis*, **13:** 1485-1489.