Full Length Research Paper

Isolation and characterization of arsenite oxidizing *Pseudomonas lubricans* and its potential use in bioremediation of wastewater

Abdul Rehman*, S. Awais Butt and Shahida Hasnain

Department of Microbiology and Molecular Genetics, University of the Punjab, New Campus, Lahore 54590, Pakistan

A bacterium, *Pseudomonas lubricans*, isolated from heavy metal laden industrial wastewater, has been shown to tolerate multiple heavy metals suggesting its importance in bioremediation of industrial effluents. *P. lubricans* tolerated As(III) up to 3 mg ml^{-1}, Cu^{2+} up to 0.7 mg ml^{-1}, Hg^{2+} up to 0.4 mg ml^{-1}, Ni^{2+} up to 0.4 mg ml^{-1} and Cr^{6+} up to 0.5 mg ml^{-1}. *P. lubricans* showed optimum growth at pH 7 while optimum temperature for growth was 30°C. *P. lubricans* could oxidize As(III) 42% (42 μg mg^{-1} of protein), 78% (78 μg mg^{-1} of protein) and 95% (95 μg mg^{-1} of protein) from the medium after 24, 48 and 72 h of incubation at optimal conditions, respectively. The arsenite oxidizing ability shown by *P. lubricans* indicates its potential application in biological treatment of wastewaters contaminated with arsenic.

Key words: Arsenic, wastewater, *Pseudomonas lubricans*, bioremediation.

INTRODUCTION

Arsenic is the most prevalent environmental toxic metal and is first on the superfund list of hazardous substances. Presently, arsenic contamination of drinking water constitutes an important public health problem in numerous countries throughout the world (Smith et al., 2002; Arriaza, 2005; Liu et al., 2009). Arsenic is a known human carcinogen (Hughes, 2002; Shi et al., 2004). Arsenic toxicity causes skin lesions and damage mucous membranes, digestive, respiratory, circulatory and nervous system. Moreover, it is associated with skin, liver and lung cancers (Wang et al., 2001). So, the World Health Organization recommends a provisional drinking water guideline of 10 ppb for arsenic.

Arsenic has been shown to induce chromosomal aberrations and sister chromatid exchanges when present during DNA replication, the results of genotoxicity studies indicated that it is mutagenic. 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, oxidation or reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies and evaporation recovery (Ahluwalia and Goyal, 2007; Kuan et al., 2009; Mouedhen et al., 2009). These processes may be ineffective or extremely expensive especially when the metals in solution are in the range of 1 - 100 mg l^{-1} (Nourbakhsh et al., 1994). Therefore, it is important to develop an innovative, low cost and eco-friendly technique for metal removal from water.

A large number of microorganisms are capable of growing in the presence of high concentrations of heavy metal (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 (up to 100 mM arsenate or up to 20 mM arsenite). Several bacteria (Cervantes et al.,

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*Correspondence author. E-mail: rehman_mmg@yahoo.com. Tel: 92-42-9231249.

Abbreviations: LB, Luria-bertani; **MIC,** minimum inhibitory concentration; **DCPIP,** dichlorophenolindophenol; **PMS,** phenazine methosilfate.

1994; Ahmed and Rehman, 2009) belonging to the genera *Acidithiobacillus*, *Bacillus*, *Deinococcus*, *Desulfito-bacterium* and *Pseudomonas* (de-Vicente et al., 1990; Dopson et al., 2001; Niggemyer et al., 2001; Suresh et al., 2004) have also been reported to be resistant to arsenic. Since heavy metals are ubiquitously present in the environment, microorganisms have developed mechanisms to resist the toxic effects of these metals (White and Gadd, 1986).

The study deals with the isolation and characterization of arsenic resistant bacterium from a contaminated environment able to oxidize arsenite and to assess this property for its exploitation in metal detoxification and environmental bioremediation.

MATERIALS AND METHODS

Sample collection and analysis

Industrial wastewater samples were collected in screw capped sterilized bottles from Sheikhupura (Pakistan). Some physicochemical parameters of wastewater namely: temperature, pH, dissolved oxygen and arsenic concentration were measured according to APHA (1989).

Isolation of arsenite 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 As(III) ml^{-1}. 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 to 7 - 7.2 and then 1.5 g agar was added in 250-ml flask. The medium was autoclaved at 121°C for 15 min. The growth of the bacterial colonies was observed after 24 h of incubation at 30°C. Effect of As (III) on the growth of bacterial isolate was determined in acetate minimal medium (Pattanapipitpaisal et al., 2001) which contained (g/l): NH$_4$Cl, 1.0; CaCl$_2$.H$_2$O, 0.001; MgSO$_4$.7H$_2$O, 0.2; FeSO$_4$ .7H$_2$O, 0.001; sodium acetate, 5; yeast extract, 0.5; K$_2$HPO$_4$, 0.5 (pH 7) supplemented with NaH$_2$AsO$_3$ (100 µg ml^{-1}). It was again incubated at 30°C for 24 h. This process was repeated with successively higher arsenite concentrations (0.1, 0.2, 0.3 and 3 mg ml^{-1}) until the minimum inhibitory concentration (MIC) of the bacterial isolate was obtained. Experiments were carried out in duplicate.

For screening the arsenite-oxidizing bacteria, the AgNO$_3$ method described by Simeonova et al. (2004) was used. Agar plates (acetate minimal medium) with NaH$_2$AsO$_3$ (100 µg ml^{-1}) were inoculated with arsenite resistant bacterial isolates, incubated at 30°C for 48 h and flooded with a solution of 0.1 M AgNO$_3$. A brownish precipitate revealed the presence of arsenate in the medium (Lett et al., 2001).

Identification of the bacterial isolate

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 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 2 min and a final extension at 72°C for 5 min. 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 bacterium, two parameters that is 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 bacterial isolate by overnight growth at 37°C in LB broth. The four sets of tubes were incubated at 25, 30, 37 and 45°C. After 12 h incubation period, their absorbance was measured 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 9 sets, each containing 3 test tubes and their pH was adjusted to 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0, then autoclaved. These tubes were inoculated with 20 µl freshly prepared culture of the bacterium. After an incubation period of 12 h, their absorbance was measured at 600 nm.

Effect of arsenite on bacterial growth

Growth curves of bacterial isolate were determined in acetate minimal medium with different concentrations of arsenite 0, 0.5, 1, 2 and 3 mg ml^{-1}. For bacterial isolate 50 ml medium was taken in one set consisting of 5 flasks, autoclaved and then inoculated with 20 µl of the freshly prepared inoculums. The cultures were incubated at 30°C in an incubator shaker at 100 rpm. An aliquot of culture was taken at regular intervals (0, 4, 8, 12, 16, 20, 24, 28, 32 and 36 h) to measure absorbance at 600 nm.

Resistance to heavy metal ions

The cross heavy metal resistance of bacterial isolate was determined by using stock solutions of 10 mgml^{-1} of different metal salts (cadmium chloride, copper sulphate, potassium dichromate, mercuric chloride and nickel chloride). The cross metal resistance was checked by increasing the concentration of respective metal in a stepwise manner with 100 µg ml^{-1} in acetate minimal medium. Culture flasks containing 100 ml medium and metal ions were inoculated with 20 µl from overnight bacterial cultures and incubated at 30°C for 24 h. Growth was measured as optical density at 600 nm.

Arsenic oxidation by bacterial isolate

The ability of bacterial isolate to oxidize arsenite was checked by adding As (III) at a concentration of 100 µg ml^{-1} in the acetate minimal medium. The control culture medium was also run for As (III) containing the same concentration as in treated one that is, 100 µg ml^{-1} but without bacterial isolate. The cultures were incubated at 30°C for 24 h and cells were harvested by centrifugation at 14000 rpm for 10 min, washed twice in 50 mM phosphate buffer (pH 7) and re-suspended in 5 ml of 50 mM phosphate buffer (pH 7). Cells were disrupted by sonication (Heilscher Ultrasonic Processors UP 400, S) at 4°C for 1 time for 15 s and centrifuged at 14000 rpm for 1 h at 4°C. The pellet re-suspended in 50 mM phosphate buffer (pH 7) represents the membrane fraction while the supernatant represents the soluble fraction. Arsenite oxidase activity was
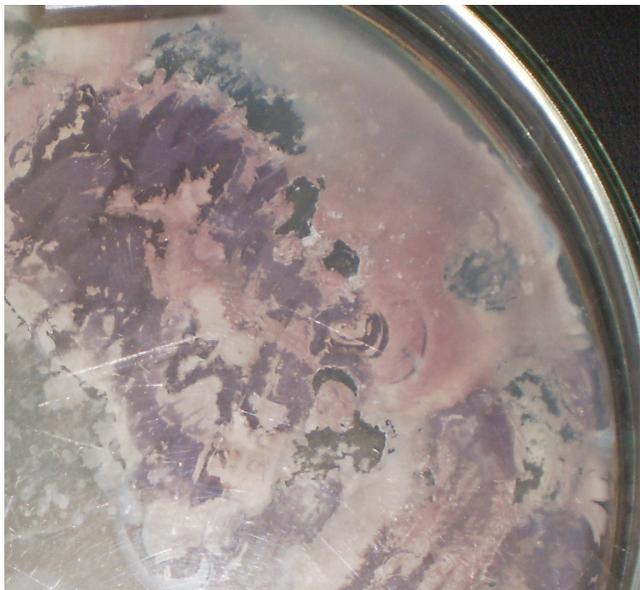
Figure 1. Appearance of brownish precipitate after the application of 0.1 M AgNO₃ in agar plate supplemented with NaH₂AsO₃ (100 μg ml⁻¹) in which *P. lubricans* was growing.

determined by transfer of reducing equivalents from As(III) 200 μM 2, 4 -dichlorophenolindophenol (DCPIP) in 50 mM phosphate buffer (pH 7) with 2mM phenazine methosilfate (PMS) after suitable time intervals (Anderson et al.,1992). The protein concentration was estimated by the Lowry method (1951) after hydrolysis of the cells in 0.4 N NaOH at 100°C for 10 min. Bovine serum albumin was used as standard.

Statistical analysis

Observations were made and all the experiments were 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 waste-water were ascertained, from where arsenic tolerant bacteria were isolated. The temperature of different samples ranged between 28 to 35°C, pH ranged between 6.4 and 8.0, dissolved oxygen between 0.42 ± 0.03 and 1.20 ± 0.01 mg l⁻¹ and As ranged between 0.80 ± 0.04 and 1.92 ± 0.03 μg ml⁻¹.

Isolation of arsenic-oxidizing bacterium

Six bacterial isolates were obtained initially when 100 μg As(III) ml⁻¹ in the medium was provided and isolation of single colonies on plates, containing high concentrations

of As(III) on which the organisms were originally isolated. Only one bacterial isolate resisted As(III) up to 3 mg ml⁻¹ and was selected for further study. When tested with AgNO₃ in agar plates, only one bacterial isolate demonstrated the ability to oxidize arsenite into arsenate. The silver nitrate test was based on the quality reaction between AgNO₃ and arsenite or arsenate ions. The interaction with As(III) generate bright yellow precipitate and with As(V) brownish precipitate (Krumova et al., 2008). In this study, *Pseudomonas lubricans* isolated from Industrial wastewater showed high arsenite oxidizing potential (Figure 1).

Bacterial identification

The partially amplified (500 bp) fragment of 16S rRNA gene from local isolate 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 BLAST query revealed that this gene is 98% homologous to *P. lubricans*. The nucleotide sequences coding for the 16S rRNA gene of *P. lubricans* have been submitted to the GenBank database under accession number EU729357. Other close matches included *P. lubricans* strain RS1 DQ842018.3, (gi/225908541); 100% similarity), *Pseudomonas* sp. KT-ql-122 FJ611930.1, (gi/ 222431647); 100% similarity), *Pseudomonas alcaliphila* strain Q1-3 EU 144361.1 (gi/ 157649155); 100% similarity) and *Pseudomonas lubricans* strain SF1 FJ600733.1 (gi/222431627); 100% similarity). Dendrogram showing (Figure 2) the percentage similarity of the bacterial isolate with different species of *Pseudomonas* is shown in Figure 2.

Optimum growth conditions

The most suitable temperature for arsenic-resistant bacterial isolate was found to be 30°C. *P. lubricans* showed maximum growth at pH 7. The growth curve pattern was studied by growing the organism in the presence of different As (III) (0, 0.5, 1, 2, 3 mg ml⁻¹). The growth pattern of the *P. lubricans* was significantly different compared with control and the growth rate of *P. lubricans* was lower in the presence of 1, 2 and 3 mg arsenite ml⁻¹. The growth pattern is shown in Figure 3.

Multiple metal resistances

P. lubricans was found to be resistant to As(III) up to a concentration of 3 mg ml⁻¹. *P. lubricans* was also checked for its resistance to other heavy metal, likes, Cd, Cr(VI), Cu, Hg and Ni. *P. lubricans* was able to resist Cd²⁺ (100 μg ml⁻¹), Cu²⁺ (700 μg ml⁻¹), Cr⁶⁺ (500 μg ml⁻¹), Hg²⁺ (400 μg ml⁻¹) and Ni²⁺ (300 μg ml⁻¹).
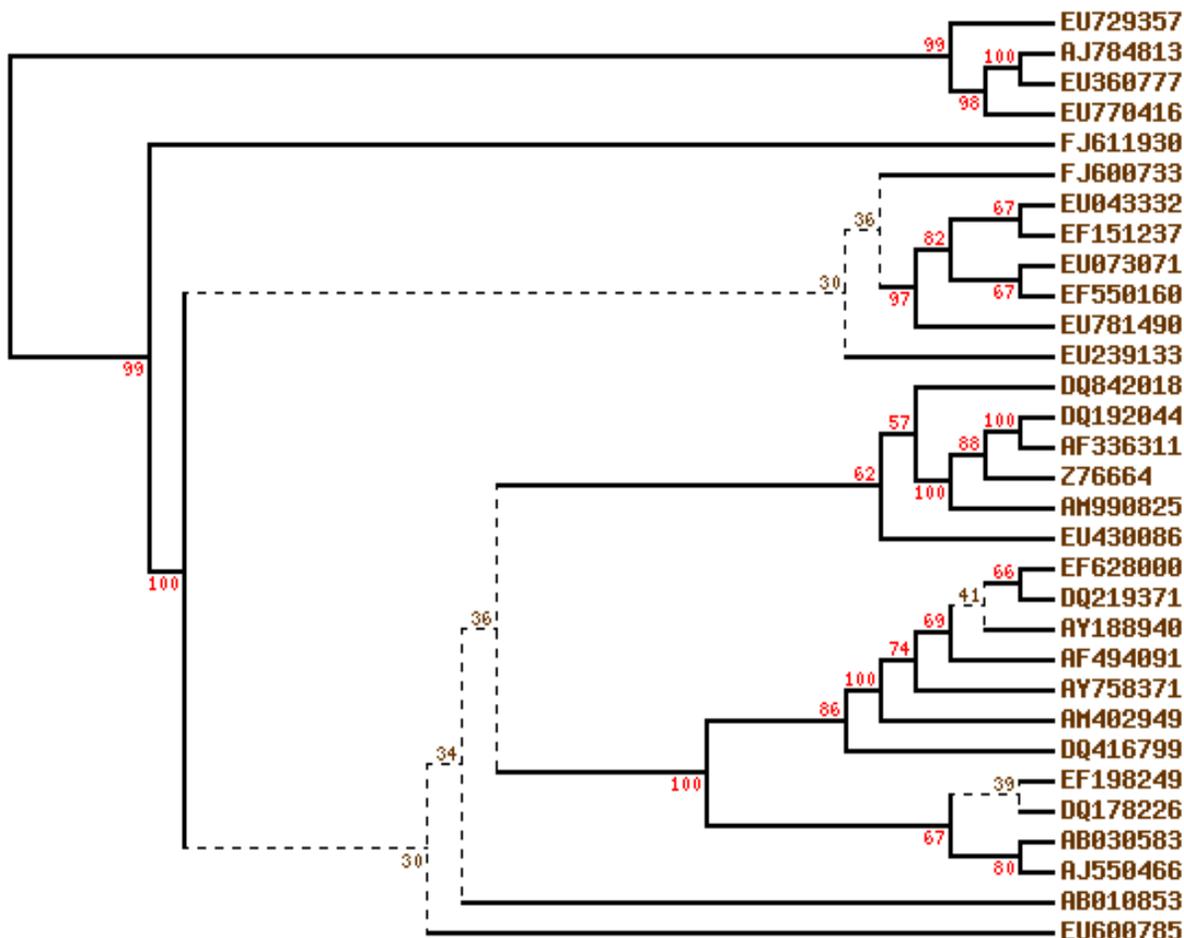
**Figure 2.** Dendrogram showing the similarity of *P. lubricans* with other close members of *Pseudomonas* sp.
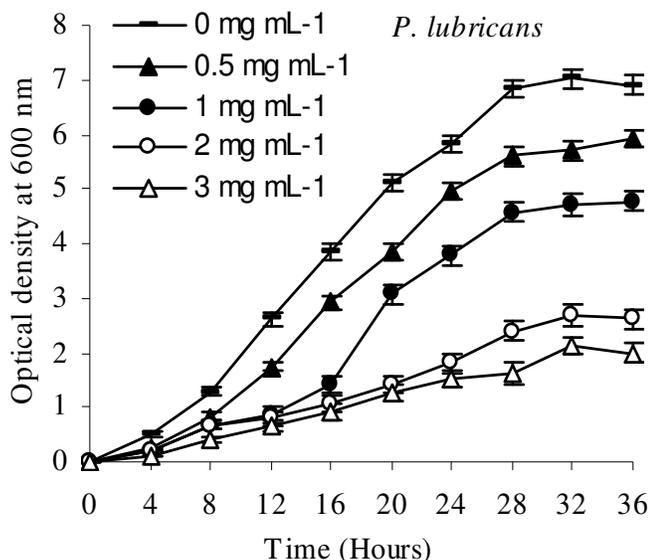
Figure 3. Effect of different arsenite concentrations (0.5, 1, 2 and 3 mg l^{-1}) on the cell growth of *P. lubricans* in acetate minimal medium after incubation at 30°C.

Arsenite-oxidation ability of *P. lubricans*

Arsenite oxidizing activity of the bacterial isolate was determined in crude extracts of bacterial cells. *P. lubricans* could oxidize 42% As(III) (42 μg mg^{-1} of protein), 78% (78 μg mg^{-1} of protein) and 95% (95μg mg^{-1} of protein) from the medium after 24, 48 and 72 h, respectively. The arsenite oxidase activity was in significant in culture without arsenite when compared with As(III)-treated culture.

DISCUSSION

A variety of mechanisms exist 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, 2008). 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,

Table 1. Arsenite resistance in different microorganisms.

Microorganisms	Arsenite (mM)	Reference
Aeromonas sp. CA1, *Exiguobacterium* sp. WK6	2 0	Anderson and Cook (2004)
Corynebacterium glutamicum	60	Mateos et al. (2006)
Aeromonas, *Bacillus*, *Pseudomonas*	16.6	Pepi et al. (2007)
Pseudomonas putida	26	Chang et al. (2007)
Thiomonas sp.	2.6	Duquesne et al. (2008)
Pseudomonas sp.	0.5	Valenzuela et al. (2009)
Pseudomonas lubricans	40	This study

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 bacterium isolated in this study was, *P. lubricans*, based on 16S rDNA sequence analysis. The genus *Pseudomonas* is composed of ubiquitous bacteria endowed with a remarkable adaptability to diverse environments. *Pseudomonas aeruginosa* strains isolated from marine environments also showed arsenic-resistance (de Vincente et al., 1990).

Arsenite resistant bacteria have also been isolated from industrial effluents by several groups (Anderson and Cook, 2004; Mateos et al., 2006; Chang et al., 2007; Duquesne et al., 2008; Valenzuela et al., 2009). In this study, *P. lubricans* was found to be highly resistant to arsenite at a concentration of 3 mg ml^{-1} (Table 1). *P. lubricans* also showed resistance against Ni^{2+}, Cu^{2+}, Cd^{2+}, Hg^2 and Cr^{6+} at a concentration of 300, 700, 100, 400 and 500 µg ml^{-1}, respectively. The order of resistance, in terms of metal concentration, was $Cu^{2+} > Cr^{6+} > Hg^{2+} > Ni^{2+} > Cd^{2+}$.

Toxic 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 specifically confer resistance to inorganic arsenic, 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).

Resistance to arsenic species in both Gram-positive and negative bacteria results from energy-dependent efflux of either arsenate or arsenite from the cell mediated through the *ars* operon (Cervantes et al., 1994; Ji and Silver, 1992a). Oxidation of As-(III), which is done by a periplasmic enzyme arsenite oxidase, represents a potential detoxification process that allows microorganisms to tolerate higher levels of arsenite. In this respect, various bacterial strains capable of oxidizing As (III) to As(V) have been reported (Mukhopadhyay et al., 2002; Chang et al., 2007; Duquesne et al., 2008; Valenzuela et

al., 2009). In this study, *P. lubricans* showed its ability to oxidize As(III) 42, 78 and 95% from the medium after 24, 48 and 72 h, respectively. The arsenite oxidase activity was insignificant in non-As (III)-treated culture as compared to the As(III)-treated culture. Arsenite-oxidizing bacteria could play an important role in both arsenic oxidation and mobilization and could be efficiently used in bioremediation of arsenic-polluted waters (Valenzuela et al., 2009).

Conclusion

The presence of arsenic in water is frequently reported and arsenite is more mobile, highly soluble and more toxic than arsenite. The most appropriate way of toxic arsenite removal is to oxidize it into arsenate which is less soluble and much more easily removed. In this study, *P. lubricans* showed, high resistance against arsenic up to 3 mg ml^{-1}. Also, resistance to Cu^{2+} (700 µg ml^{-1}), Hg^{2+} (400 µg ml^{-1}), Ni^{2+} (400 µg ml^{-1}) and Cr^{6+} (500 µg ml^{-1}) was noted. *P. lubricans* could oxidize As(III) 42, 78 and 95% from the medium after 24, 48 and 72 h, respectively. Therefore *P. lubricans* may be applicable for the treatment of industrial effluents contaminated with arsenic.

REFERENCES

Ahluwalia SS, Goyal D (2007). Microbial and plant derived biomass for removal of heavy metals from wastewater. Bioresour. Technol. 98: 2243-2257.

Ahmed A, Rehman A (2009). Isolation of Cr (VI) reducing bacteria from industrial effluents and their potential use in bioremediation of chromium containing wastewater. J. Environ. Sci. 21: 814-820.

Anderson CR, Cook GM (2004). Isolation and characterization of arsenate-reducing bacteria from arsenic contaminated sites in New Zealand. Curr. Microbiol. 48: 341-347.

Anderson GL, Williams J, Hille R (1992). The purification and characterization of arsenite oxidase from *Alcaligenes faecalis*, a molybdenum-containing hydroxylase. J. Biol. Chem. 267: 236741-23682.

APHA (1989). Standard Methods for the Examination of Water and Wastewater. APHA, 18th ed. Washington, DC.

Arriaza B (2005). Arseniasis as an environmental hypothetical explanation for the origin of the oldest artificial mummification practice in the world. Chungara, 37: 255-260.

Ballatori N (1994). Glutathione mercaptides as transport forms of metals. Adv. Pharmacol. 27: 271-298.

Cervantes C, Ji G, Ramirez JL, Silver S (1994). Resistance to arsenic compounds in microorganisms. FEMS Microbiol. Rev. 15: 355-367.

Chang J-S, Yoon I-H, Kim K-W (2007). Isolation and *ars* detoxification of arsenite-oxidizing bacteria from abandoned arsenic-contaminated mines. J. Microbiol. Biotechnol. 17: 812-821.

Chou WC, Hawkins AL, Barrett JF, Griffin CA, Dang CV (2001). Arsenic inhibition of telomerase transcription leads to genetic instability. J. Clin. Invest. 108: 1541-1547.

Duquesne K, Lieutaud A, Ratouchniak J, Muller D, Lett M-C, Bonnefoy V (2008). Arsenic oxidation by a chemoautotrophic moderately acidophilic *Thiomonas* sp.: from the strain isolation to the gene study. Environ. Microbiol. 10(1): 228-237.

De-Vicente A, Aviles M, Codina JC, Borrego JJ, 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 EB, Hallberg KB (2001). Chromosomally encoded arsenical resistance of the moderately thermophilic acidophile *Acidithiobacillus caldus*. Extremophiles, 5: 247-255.

Gaballa A, Helmann JD (3003). *Bacillus subtilis* CPx-type ATPases: Characterization of Cd, Zn, Co and Cu efflux systems. Biometals, 16: 497-505.

Gadd GM (1990). Heavy metal accumulation by bacteria and other microorganisms. Experientia, 46: 834-840.

Holan ZR, Volesky B (1995). Accumulation of Cd, lead and nickel by fungal and wood biosorbents. Appl. Biochem. Biotechnol. 53: 133-146.

Hughes MF (2002). Arsenic toxicity and potential mechanisms of action. Toxicol. Lett. 133: 1-16.

Ji G, Silver S (1992a). Regulation and expression of the arsenic resistance operon of *Staphylococcus aureus* plasmid p. l258. J. Bacteriol. 174: 3684-3694.

Krumova K, Nikolovska M, Groudeva V (2008). Isolation and identification of arsenic-transforming bacteria from arsenic contaminated sites in Bulgaria. Biotechnol. Biotechnol. Equip. 22(2): 721-728.

Kuan WH, Hu CY, Chiang MC (2009). Treatment of As (V) and As (III) by electrocoagulation using Al and Fe electrode. Water Sci. Technol. 60(5): 1341-1346.

Lett M-C, Paknikar K, Lievremont D (2001). A simple and rapid method for arsenite and arsenate speciation. In: Ciminelli VST, Garcia O, (eds), Biohydrometametallurgy-fundamentals, technology and sustainable development. Part B. Elsevier Sci., New York.

Liu C-P, Luo C-L, Gao Y, Li F-B, Lin L-W, Wu C-A, Li X-D (2009). Arsenic contamination and potential health risk implications at an abandoned tungsten mine, southern China. Environ. Pollut. Doi: 10.1016/j.envpol.2009.09.029.

Lovely DR, Coates JD (1997). Bioremediation of metal contamination. Curr. Opin. Biotechnol. 8: 285-289.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement wih the Folin phenol reagent. J. Biol. Chem. 193: 265 275.

Mateos LM, Ordonez E, Letek M, Gil JA (2006). *Corynebacterium glutamicum* as a model bacterium for the bioremediation of arsenic. Int. Microbiol. 9: 207-215.

Matsui M, Nishigori C, Toyokuni S, Takada J, Akaboshi M, Ishikawa M, Imamura S, 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.

Mouedhen G, Feki M, De Petris-Wery M, Ayedi HF (2009). Electrochemical removal of Cr (VI) from aqueous media using iron and aluminum as electrode materials: towards a better understanding of the involved phenomena. J. Hazard. Mater. 168(2-3): 983-991.

Mukhopadhyay R, Rosen BP, Phung LT, Silver S (2002). Microbial arsenic: from geocycles to genes and enzymes. FEMS Microbiol. Rev. 26: 311-325.

Nies DH (1992). Resistance to cadmium, cobalt, zinc and nickel in microbes. Plasmid, 27: 17-28.

Niggemyer A, Spring S, Stackebrandt E, Rosenzweig RF (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, Calgar A (1994). A comparative study of various biosorbents for removal of chromium (ε) ions from industrial wastewater. Process Biochem. 29: 1-5.

Pattanapipitpaisal P, Mabbett AN, Finlay JA, Beswick AJ, Paterson-Beedle M, Essa A, Wright J, Tolley MR, Badar U, Ahmed N, Hobman JL, Brown NL, Macaskie LE (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 NL, Macaskie LE (2001). Chromate reduction and 16S rRNA identification of bacteria isolated from a Cr (VI)-contaminated site. Appl. Microbiol. Biotechnol. 57: 257-261.

Pepi M, Volterrani M, Renzi M, Marvasi M, Gasperini S, Franchi E, Focardi SE (2007). Arsenic-resistant bacteria isolated from contaminated sediments of the Orbetello Lagoon, Italy and their characterization. J. Appl. Microbiol. 103: 2299-2308.

Rehman A, Ali A, Muneer B, Shakoori AR (2007). Resistance and biosorption of mercury by bacteria isolated from industrial effluents. Pak. J. Zool. 39(3): 137-146.

Rehman A, Shakoori FR, Shakoori AR (2007). Heavy metal resistant *Distigma proteus* (Euglenophyta) isolated from industrial effluents and its possible role in bioremediation of contaminated wastewaters. World J. Microbiol. Biotechnol. 23: 753-758.

Rehman A, Shakoori FR, Shakoori AR (2008). Heavy metal resistant freshwater ciliate, *Euplotes mutabilis*, isolated from industrial effluents has potential to decontaminate wastewater of toxic metals. Bioresour. Technol. 99: 3890-3895.

Rosen BP (1999). Families of arsenic transporters. Trends Microbiol. 7: 207-212.

Silver S, Phung LT (1996). Bacterial heavy metal resistance: new surprises. Ann. Rev. Microbiol. 50: 753-789.

Silver S, Phung LT (2005). Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. Appl. Environ. Microbiol. 71: 599-608.

Simeonova D, Lievremont D, Lagarde F, Muller D, Groudeva V, Lett M-C (2004). Microplate screening assay for detection of arsenite-oxidizing and arsenate-reducing bacteria. FEMS Microbiol. Lett. 237: 249-253.

Smith AH, Lopipero PA, Bates MN, Steinmaus CM (2002). Public health. Arsenic epidemiology and drinking water standards. Science, 296: 2145-2146.

Shi H, Shi X, Liu K-J (2004). Oxidative mechanisms of arsenic toxicity and carcinogenesis. Mol. Cell. Biochem. 255: 67-78.

Suresh K, Reddy GSN, Sengupta S, 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 TS, Hsu TY, Chung CH, Wang AS, Bau DT, Jan KY (2001). Arsenite induces oxidative DNA adducts and DNA-protein cross-links in mammalian cells. Free Radical Biol. Med. 31: 321-330.

White C, 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 LM, Jaspers I, Devlin RB, Reed W, Samet JM (1999). Activation of the EGF receptor signaling pathway in human airway epithelial cells exposed to metals. Am. J. Physiol. 277: 924-931.

Valenzuela C, Campos VL, Yanez J, Zaror CA, Mondaca MA (2009). Isolation of arsenite-oxidizing bacteria from arsenic-enriched sediments from Camarones River, Northern Chile. Bull. Environ. Contam. Toxicol. 82: 593-596.

Zhitkovitch A, Costa M (1992). A simple sensitivity assay to detect DNA-protein crosslinks in intact cells and *in vivo*. Carcinogenesis, 13: 1485-1489.