Accumulation of some heavy metals by metal resistant avirulent *Bacillus anthracis* PS2010 isolated from Egypt

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The bacteria with a high growth rate were isolated from polluted industrial waste water. The bacteria *Bacillus anthracis* PS2010 have variable resistant to heavy metals such as Cd, Cu, Co, Zn and Pb. Out of which the minimal inhibitory concentrations were 0.6, 2.0, 0.8, 4.0 and 3.0 mM, respectively. The potent bacterium has optimal biosorption capacity raised according to the metal, incubation temperature, pH of the solution and contact time. Under optimal conditions, the bacterium was capable of taking up the heavy metals Cd, Cu, Co, Zn and Pb at 3.41, 2.03, 4.75, 5.22 and 6.44 mg/g dry weight. Transmission electron microscopy showed accumulation of Pb metal external to bacterial cells. The mechanism of heavy metal tolerance in *Bacillus anthracis* PS2010 is chromosomally encoded. *Bacillus anthracis* harbored no plasmid.

Key words: Heavy metal uptake, bacterial biosorption, plasmid, *Bacillus anthracis* PS2010.

INTRODUCTION

Mobilization of heavy metals in the environment due to industrial activities is of serious concern due to the toxicity of these metals in humans and other forms of life. Removal of toxic heavy metals from industrial waste waters is essential from the standpoint of environmental pollution control (Puranik and Pakniker, 1999; Guangyu and Thiruvenkatachari, 2003). Heavy metals mercury (Hg), nickel (Ni), lead (Pb), arsenic (As), zinc (Zn), cadmium (Cd), aluminum (Al), platinum (Pt), copper (Cu) and cobalt (Co) are trace metals with a density of at least five times that of water, they are stable elements (meaning they cannot be metabolized by the body) and bio-accumulative (passed up the food chain to humans). These include: Hg, Ni, Pb, As, Zn, Cd, Al, Pt, Cu and Co. Some heavy metals have function in the body while others can be highly toxic for human health (Parry, 2009; Hornung et al., 2009). Toxicity of metallic ions could be the result of competition with or replacing a functional metal as well as causing conformational modification, denaturation, and inactivation of enzymes and disruption of cellular and organelles integrity (Blackwell et al., 1995).

Remediation technologies using microorganisms are feasible alternatives to the physical cleaning of soil or the concentration of metals in polluted water by physical or chemical means (Valls and de Lorenzo, 2002; Abou Zeid et al., 2009; Adewole et al., 2010). Metal tolerance reflects the ability of an organism to survive in an environment with high concentration of metals or to accumulate high concentration of metal without dying. Metal exposure also leads to the establishment of tolerant microbial populations,

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which are often represented by several Gram positives belonging to Bacillus, Arthrobacter and Corynebacterium, as well as Gram negatives as Pseudomonas, Alcaligenes, Ralstonia and Burkholderia (Kozdro and Van Elsas, 2001; Ellis et al., 2003; Ajaz et al., 2010). In contaminated sites, these populations may be involved in the alteration of mobility of metals through their reduction, accumulation, and in situ immobilization by extracellular precipitation (Roane, 1999). Different microorganisms such as fungi, yeast and bacteria were tested for the availability and biosorption potential to bind heavy metals (Volensk and Holan, 1995). There are at least three types of microbial processes that can influence toxicity and transport of metals and radionuclide’s: biotransformation, bioaccumulation and biodegradation. However, microorganisms can interact with these contaminants and transform them from one chemical form to another by changing their oxidation state through the addition (reduction) or removal (oxidation) of electrons. Several authors have reported the high capability of heavy metals bioaccumulation by Gram negative bacteria (Noghabi et al., 2007; Choi et al., 2009; El-Shanshoury et al. 2012). Bacterial resistance to heavy metals might be chromosomal or plasmid mediated (Raval et al., 2000; Zouboulis et al., 2003). Zolgharnein et al. (2007) reported that the frequency of the occurrence of plasmids in heavy metal resistant bacteria was more than that in common bacteria.

The main objective of this study was to obtain a local bacterium resistant to heavy metals, in order to be used for remediation of metal ions in polluted habitats in Egypt. In this connection, the potential of Bacillus anthracis PS2010 to absorb and uptake Cd, Cu, Co, Zn, and Pb was focused on, with special emphasis on Pb. The mechanism and the form of Pb accumulation by the bacterium are discussed.

MATERIALS AND METHODS

Source of bacterial isolate

The tested isolate, B. anthracis PS2010 (accession no. HQ856038) used in this study was isolated previously from location polluted with heavy metals in Egypt. The isolate was identified by sequencing 16S rRNA gene and comparing the sequences with database library using analysis software. The program Blast was used to assess the DNA similarities and multiple sequence alignment and molecular phylogeny was performed using BioEdit software (Hall, 1999).

Samples collection

Samples were collected from 3 different polluted locations: a lathe and motor manufacturing workshops in Tanta, El-Gharbia Governorate, and industrial wastewater of Industrial Area in Quesna, El-Monofeya Governorate. The samples included dusts containing metal filings, grinding metals and industrial wastewater.

Isolation of bacteria

The isolation of bacteria was carried out on nutrient agar medium adjusted at pH 7.3 ± 0.2. One gram of each dry contaminated dust was dissolved in 50 ml sterile distilled water, and then 250 µl from the resultant suspension were spread on the surface of nutrient plates. The plates were incubated for 24 h at 35°C. Grown colonies were investigated for their morphological characteristics, purified and kept at 5°C as slant cultures.

Isolation and selection of metals resistance bacteria

All the isolated bacteria were checked for their metal tolerance against five selected metals: Cd, Cu, Co, Zn and Pb. Aqueous solutions of the metal salts: CdCl2-2H2O, CuSO4-5H2O, CoCl2, ZnSO4-7H2O and Pb(NO3)2 were prepared in de-ionized water and 0.1 mM/L were added to the nutrient agar medium. Media were sterilized, dispensed in Petri plates and then inoculated with bacteria; the plates were incubated at 35°C for 24 h. The tolerance was checked on the basis of growth observed within 24 h of cultivation according to Chowdhury et al. (2008). The grown colonies were used for detection of minimal inhibitory concentrations (MICs) for each metal. The most highly tolerant bacterium (with highest MICs) was selected for identification and further study.

Preparation of bacterial culture

One liter of nutrient broth medium free of metals was prepared and sterilized by autoclaving. Medium was inoculated with 24-h-old B. anthracis PS2010 culture previously prepared and grown to the end of exponential phase (growth curve data not shown).

Determination of the minimum inhibition concentration (MIC)

The minimum inhibition concentration was checked for its metal tolerance against five selected metal salts: CdCl2-2H2O, CuSO4-5H2O, CoCl2, ZnSO4-7H2O, and Pb(NO3)2. Aqueous solutions of these salts were prepared in de-ionized water, the pH values of the solutions were adjusted to neutral (pH 7). The flasks containing media and variable concentration of metals were incubated at 35°C with B. anthracis PS2010 for 24 h. The highly tolerant isolate (with the highest MICs values) was selected for characterization, identification and further experiments.

Electron microscopy

The highly uptake isolate (of the five metal ions mg/g dry weight), (with following order: Pb2+ > Zn2+ > Co2+ > Cd2+ > Cu2+) were selected for characterization, identification. Before and after the treatment with Pb(NO3)2, cell of isolate was examined by scanning electron microscope (SEM) to detect any change in the morphology of the cells as a result of metal treatment. The control and treated cultures were also examined by transmission electron microscopy (TEM) in order to identify the location of lead particles within the cells (Chowdhury et al., 2008). Cells of control and treated cultures (as described before) were centrifuged at 5000 rpm, washed twice and fixed in 2.5% buffered glutaraldehyde in 0.1 M PBS (phosphate buffer solution) pH: 7.4 for 24 h at 4°C, washed three times with PBS for 10 min each time and then centrifuged at 5000 rpm. These steps were followed by post fixation in 1% osmic acid for 30 min. It was dehydrated in a series of ethyl-alcohol (30 to 100%) infiltrated with acetone each concentration for 30 min.

Transmission electron microscopy (TEM)

After dehydration, samples were embedded in Araldite resin. The plastic molds were cut at 850 nm thicknesses in a LEICA Ultra cut ultra-microtome, and stained with 1% toluidine blue. After examination of semi-thin sections, ultrathin sections were cut at thickness of...
75 nm, stained with uronyl acetate for 45 min, then counter stained with lead citrate and examined. Images were taken using a JEOL, JEM-100 SX electron microscope. All the isolated bacteria that were able to grow on media supplemented with different salt decreased gradually by increasing the concentration of each metal salt. The isolate was low tolerant to all metals which showed higher tolerance, in comparison with other isolates. The highly tolerant organism for most metals was selected for characterization, identification and further experiments.

Penicillin sensitivity
The penicillin sensitivity of the isolate was observed according to Mueller-Hinton (1941). Mueller Hinton agar medium was prepared and sterilized in autoclave at 121°C for 20 min. The medium was suspended in sterilized Petri plates prior to inoculation of the plates with the tested organism and then a sterilized penicillin disc (Bioanalysis, 10 U) was placed in the centre of the plate. The plate was incubated overnight at 35°C. The presence of inhibition zone around the disc was checked.

Sequencing of 16S-rRNA gene and phylogenetic analysis
The bacterium selected as the most resistant isolate to all five heavy metals was identified and confirmed using Biolog automated system Bochner (1989). The selected isolate was identified by sequencing of 16Sr-RNA gene. Bacterial genomic DNA was extracted from the cells by using Qiagen kit. The DNA was used as template for PCR using universal primers. The forward primer is 5'-AAC TGG AGG AAG GTG GGG AT-3', The purified product of the PCR is used as template in cycle sequencing using 3130 X / Genetic Analyzer, Applied Biosystems, Hitachi, Japan, with Big dye terminator cycle sequences technique, developed by Sanger et al. (1977). The products were purified using special column. The sample became ready for sequencing in 3130 X DNA sequencer and analysis. Blast program was used to assess the DNA similarities and multiple sequence alignment and molecular phylogeny were performed using BioEdit software (Hall, 1999).

Plasmid isolation
To prove the tested organism is avirulent, the plasmid DNA of the selected isolate was extracted, purified and separated using agarose gel electrophoresis according to the method employed by Manniatis et al. (1982), for isolation and screening plasmid. The developing bands were compared with DNA marker.

Pathogenicity of the isolated Bacillus anthracis PS2010
In order to detect if our isolated B. anthracis PS2010 was pathogenic strain or not, the presence of plasmid(s) coding for the pathogenicity was tested. Plasmid isolation was carried out in The City for Scientific Research and Biotechnology Applications, New Borg EL-Arab City, Alexandria, Egypt. The plasmid was tested by using Qiagen kit, the QIAprep miniprep procedures use the modified alkaline lysis method of Birnboim and Doly (1979), followed by adsorption of DNA onto silica in the presence of high salt.

Optimization of metal uptake
Effect of different incubation temperature
One milliliter of the aliquots of B. anthracis PS2010 selected isolate suspension, 10 h old (exponential phase) were inoculated in 100 ml nutrient broth media containing sub-MICs concentrations of CdCl2, 2H2O, CuSO4-5H2O, CoCl2, ZnSO4-7H2O, and Pb(NO3)2 respectively. After the addition of metal solutions, the media was adjusted at pH=7 by using 0.1 N NaOH and 0.1 N HCl and (0.1 N HNO3 with Pb(NO3)2. The cultures were incubated at different temperatures (25, 35 and 45°C) for 24 h. The incubated cultures were centrifuged at 5000 rpm for 20 min. The supernatants were used for the determination of the residual metal ion contents by using atomic absorption spectrophotometer (Perkin Elmer 2380) with hollow cathode lamp at specific wavelength for each metal. Control cultures without the inoculation of bacteria were prepared to detect the initial metal concentration.

Effect of different pH values
To test the pH effect of nutrient broth media containing metal solutions, the solution pH were adjusted at different values (2, 5, 7, 8 and 9). All cultures were incubated at 35°C for 24 h. The initial and the residual metal concentrations were measured.

Effect of contact times
Media containing metal solutions adjusted at pH=7 and inoculated with selected isolate was incubated at 35°C for different periods (12, 18, 24 and 48 h). The initial and residual concentrations were measured as mentioned earlier.

Determination of metal uptake by the resistant bacteria
The uptake of Cd, Cu, Co, Zn and Pb metals in mg/g dry wt. were detected. According to each metal, bacterial culture (10 h old) was adjusted at the optimal pH, incubated temperatures and optimal period of time. The cultures were centrifuged at 5000 rpm for 20 min. The supernatants were discarded and the residual bacterial pellets were washed with sterilized distilled water and then the bacterial biomass were transferred to known weight. The supernatants were used for the determination of the residual metal ions contents in mg/L. The initial metal ions contents in mg-l-1 were determined in control without bacterial cell. Supernatants were passed through bacterial filters (0.22 µm diameter). The determinations were undertaken by using Atomic Absorption spectrophotometer (model Perkin Elmer 2380) (Abou Zeid et al., 2009). The metal uptake in mg/g dry wt. was calculated according to the equation of Volesky and May-Phillips (1995):

Metal uptake (mg/g) = V (C1 - Cf) / W

Where, C1 = initial metal concentration (mg/L), Cf = final metal concentration (mg/L), V = volume of reaction (L), W = total biomass (g).

Statistical analysis
The statistical analysis was carried out using SAS program version 6.12. Data obtained were analyzed statistically to determine the degree of significance between treatments using one way analysis of variance (ANOVA) by the methods described by Cochran and Cox (1960).

RESULTS AND DISCUSSION
The pure isolated strain obtained from the polluted location was studied. Different concentrations of each metal solution were prepared, the minimum concentration of each metal added was 0.1 mM/L and the concentration was gradually increased till MIC was achieved. The isolated strain was found to give low tolerance with CdCl2 and
was found to be highly tolerant to ZnSO$_4$-7H$_2$O. The MICs of Cd$^{2+}$, Cu$^{2+}$, Co$^{2+}$, Zn$^{2+}$ and Pb$^{2+}$ were 0.6, 2.0, 0.8, 4.0 and 3.0 mM/L, respectively. This varying response of tested bacteria might be due to variation in resistance mechanisms (Abou Zeid et al., 2009).

For phylogenetic analysis, the 16S rRNA gene sequence of a single band of MW (~320 bp) was obtained (Figure 1a) when compared with those retrieved from Gen Bank database. The sequences have high similarity or are even identical to cultivable bacterial organism. The phylogenetic analysis of the 16SrRNA gene partial sequence of isolated strain revealed close similarity with *B. anthracis* TC-3, *B. anthracis* 002A48, *Bacillus thuringiensis* BMB171 and *Bacillus cereus* LS24 (96% similarity) (Figure 2).

Wang and Chen (2006), reported that the members of *B. cereus* group share many of their biochemical, morphological and they are very closely related in gene sequence based on their 16S rRNA. According to Health Protection Agency in UK (2007), for the identification of *Bacillus* species, the differentiation between *B. cereus* members depends on 3 main tests: penicillin sensitivity, motility and hemolytic activity. Since our isolate was penicillin sensitive, non-motile, with non-hemolytic activity and characteristic grayish white colonies on blood agar (Figure 3), it was identified as *B. anthracis*.

Resistance to heavy metals might be chromosomal or plasmid mediated (Gupta et al., 1999). Zolgharnein et al. (2007) reported that the frequency of the occurrence of plasmids in heavy metal resistant bacteria was more than that in common bacteria. So, it is important to get safe bacteria for possible application in metal bioremediation. Virulent strains of *B. anthracis* harbor two endogenous plasmids, pXO1 and pXO2 which code for the major known virulence factors of this organism (Thorne, 1985).

Figure 1b revealed the absence of plasmid DNA in extracts, indicating that this strain was avirulent. A virulent *B. anthracis* strain which lack these plasmids (pXO1, pXO2) have also been found and they appear to be very similar to *B. cereus* and other related species unless tests for bacteriophage susceptibility, motility and hemolysis are preformed (Henderson et al., 1994). The *B. anthracis* strain used in this study found to lack both plasmids (pXO1, pXO2), thus it was regarded as avirulent strain and safe for bioremediation purposes. It was thus submitted to Genbank as *B. anthracis* PS2010 with accession no. HQ856038. In agreement, Silver (1996), reported that bacterial cells encoded resistance systems for several toxic metal ions including Ag$^+$, As$^{3+}$, Cd$^{2+}$, Cr$^{3+}$, Cu$^{2+}$, Hg$^{2+}$, Pb$^{2+}$, Sb$^{3+}$, Te$^{2+}$ and Zn$^{2+}$. Resistance to heavy metals might be mediated by genes encoded on chromosomes, plasmids or transposons (Tenover and McGowan, 1996; Ghosh et al., 2000). These chromosomes carried genes responsible for resistance to high levels of toxic heavy metals (As$^{3+}$, Cr$^{3+}$, Cd$^{2+}$ and Hg$^{2+}$) as well as ampicillin antibiotic. The ability to grow in the presence of Pb$^{2+}$ was seen in chromosome encoded (Wasi et al., 2008).

The capacity of living cells to remove metal ions from aqueous solutions is also influenced by environmental growth conditions, as temperature, pH and biomass concentrations (Chen and Ting, 1995). In the present study, the growth and metal uptake capability of the resistant *B. anthracis* PS2010 were affected by the different environmental conditions (incubation temperature, pH value and contact time). The effect of different incubation temperatures on the uptake of the five selected metals (Figure 4) revealed that the maximum uptake for Zn$^{2+}$ and Pb$^{2+}$ was obtained at 35 and 25°C, respectively. The uptake of Zn$^{2+}$
and Pb²⁺ (%) decreased by increasing temperature. For Cd²⁺, Cu²⁺ and Co²⁺ it was clear that there is no great difference in their uptake between 25 and 35°C while the uptake was greatly decreased by increasing the incuba-
tion temperature to 45°C. Higher temperatures usually enhance sorption due to the increased surface activity and kinetic energy of the solute which could promote the active uptake or attachment of metal to cell surface, respectively (Sağ and Kutsal, 2000; Vijayaraghavan and Yun, 2007). The accumulation of heavy metals by *B. anthracis* PS2010 was found to be decreased by increasing the temperatures to 45°C, these results agree with the results obtained by Mameri et al. (1999), Prescott et al. (2002) and Uslu and Tanyol (2006).

The pH value is one of the main factors in the biosorption efficiency and binding to microorganisms (Babich and Stotzky, 1985; Lopez et al., 2000; Jalali et al., 2002; Pardo et al., 2003). Results indicate that pH 8 was optimum for Cd^{2+}, Co^{2+} and pH range 7-8 was the optimum for Cu^{2+} uptake. These results agree with that of Remacle (1990). The uptake of Co^{2+} decreased by increasing the contact period between bacteria and metal more than 18 h. The result was also obtained for *B. anthracis* PS2010 by El-Shanshoury et al. (2012).

Cell age is considered as an important factor that affects metal accumulation. During the detection of metal uptake with *B. anthracis* PS2010 illustrated in Figure 7, it was found that Pb^{2+} was the most highly uptake element while the uptake of Cu^{2+} was the lowest for the considered heavy metals. The uptake of the five metals by *B. anthracis* PS2010 was in the following order Pb^{2+} > Zn^{2+} > Co^{2+} > Cd^{2+} > Cu^{2+} with different uptake values of 6.44±0.63, 5.22±0.41, 4.75±0.39, 3.41±0.47 and 2.03±0.30 mg·g⁻¹ dry weight, respectively. This difference in the uptake may be due to the difference in mechanisms by which the bacteria can tolerate the different heavy metals.

The synthesis of Pb nanoparticles by *B. anthracis* PS2010 was detected by examining the cells of *B. anthracis* before and after treatment with 0.4 mM of Pb(NO₃)₂, with TEM. The bacterium was able to synthesize nanostructure particles from Pb (Figure 8), it was clear that these nanoparticles were synthesized extracellularly as a result of lead exposure. The X-ray powder diffraction (XRD) analysis of the dried Pb(NO₃)₂-treated cells indicated the synthesis of lead oxide (PbS) nanoparticles by *B. anthracis* PS2010 (Figure 9). The suggested mechanism for the formation of PbS nanoparticles by *B. anthracis* PS2010 occur in an aerobic condition.
Figure 4. Effect of different temperatures on heavy metal accumulation by *B. anthracis* PS2010.

Figure 5. Effect of different pH values on heavy metal accumulation by *B. anthracis* PS2010.

Under these conditions, the production and accumulation of large amounts of sulfide likely occur, which transfer across the membrane into the culture medium and can be used as sulfur source in the formation of PbS nanoparticles. Engels et al. (2000) and Rudzinski et al. (2004) reported that methanethiol under aerobic conditions is converted rapidly to dimethyldisulfide (DMDS) and/or dimethyl-trisulfide which caused precipitation for PbS
Figure 6. Effect of different contact periods on heavy metal accumulation by B. anthracis PS2010.

Figure 7. Metals uptake by B. anthracis PS2010 under the optimum conditions.
nanoparticles. Gong et al. (2007) obtained PbS nanoparticles by Desulfotomaculum sp. (strictly anaerobic sulfate-reducing bacteria). This bacterium can utilize sulfate as a terminal electron acceptor in their anaerobic oxidation of organic substrates. As a result, they produce and accumulate large amounts of sulfide which transfer across the membrane into the culture medium and could be used as sulfur source in the formation of PbS nanoparticles. This property of metal particle generation enables the bacteria to work as a living factory and as an inexpensive system to produce metal nanoparticles which have a strong application in the field of material science (Chowdhury et al., 2008). The mechanism of PbS synthesis by B. anthracis PS2010 is suggested to be a precipitation of Pb by DMDS off gas produced by the cells from methionine amino acid in the form of PbS nanoparticles (Macaskie et al., 2007).

REFERENCES

Abou Zeid AA, Hassanein AW, Hedayat SM, Fahd GAA (2009). Biosorption of Some Heavy Metal Ions Using Bacterial Species Isolated from Agriculture Waste Water Drains in Egypt. J. Appl. Sci. Res. 5(4): 372-383.

Adewole G M, Adewale T M, Ufuoma E (2010). Environmental aspect of oil and water-based drilling muds and cuttings from Dibi and Ewan off-shore wells in the Niger Delta, Nigeria. Afr. J. Environ. Sci. Technol. 4(5): 284-292.
Ajaz HM, Arasuc RT, Narayananb VKR, Zahir HMI (2010). Bioremediation of Heavy metal contaminated soil by the Exigobacterium and Accumulation of Cd, Ni, Zn and Cu from Soil environment. Inter. J. Biotechnol. 2(1): 101-107.

Babich H, Stotzky G (1985). Heavy metal toxicity to microbe-mediated ecological processes: A review and application to regulatory policies. Environ. Res. (36): 111 - 137.

Blackwell KJ, Singleton I, Tobin JM (1995). Metal cation uptake by yeast: A review. Appl. Microbial. Biotechnol. (43): 571-84.

Bochner BR (1989). Sleuthing out bacterial identities. Nature 339: 157-158.

Chang JS, Law R, Chang C (1997). Biosorption of lead, copper and cadmium by biomass of Pseudomonas aeruginosa PU 21. Water Res. 31:1651-1658.

Chen P, Ting YP (1995). Effect of heavy metal uptake on the electrokinetic properties of Saccharomyces cerevisiae. Biotechnol. Lett. 17(1):107-12.

Choi J, Lee JY, Yang J (2009). Biosorption of heavy metals and uranium by starfish and Pseudomonas putida. J. Hazard. Mater. 161(1): 157-162.

Chowdhury S, Mishra M, Adarsh VK, Mukherjee A, Thakur AR, Chauthu DN, Srivastava J (2008). Novel metal accumulator and protease secreting microbes from East Calcutta Wetland. Am. J. Biochem. Biotechnol. 4(3): 255-264.

Cochran WG, Cox GM (1960). Experimental Designs, 2nd ed. John Wiley, N.Y. 293-316.

Engels WJM, Alting AC, Arntz MMT, Gruppen H, Voragen AGJ, Smit E. (1989). Sleuthing out bacterial identities. Nature 339:157-162.

Ghosh A, Singh A, Ramteke P W, Singh V P (2000). Characterization of large plasmids encoding resistance to toxic heavy metals in Salmonella abortusequi. Biochem. Biophys. Res. Commun. 272(1): 6-11.

Gong J, Zhang Z, Bai H, Yang G (2007). Microbiological synthesis of nanophase PbS by Desulfotomaculum sp. Sci China Ser. E-Tech. Sci. 50(3): 302-307.

Guangyu Y, Thiruvenkatachari V (2003). Heavy metals removal from aqueous solution by fungus Mucor rouxii. Water Res. 37(18): 4486-4496.

Gupta A, Phung L, Chakravarty L, Silver S (1999). Mercury resistance in Bacillus cereus RC607: Transcriptional organization and two new open reading frames. J. Bacteriol. 181:7080-7086.

Hall TA (1999). BioEdit: A user-friendly biological sequence alignmenteditor and analysis program for Windows 95/NT. Nucleic Acid Symp. Ser. 41: 95-98.

Health Protection Agency (2007). Identification of Bacillus species. National Standard Method BSOP. Standards unit, Evaluations and Standards Laboratory, Centre for infection. 21(1): 1-15.

Henderson I, Duggleby CJ, Turnbull PCB (1994). Differentiation of Bacillus anthracis from other Bacillus cereus group bacteria with the PCR. Int. J. Syst. Bacteriol. (44):99-105.

Hornung RW, Lanphear BP, Dietrich KN (2009). Age of greatest susceptibility to childhood lead exposure: a new statistical approach. Environ. Health Perspect. 117(8):1309-12.

Jalali R, Ghafourian H, Sepehr S (2002). Removal and recovery of lead from copper mine effluent using non-living biomass of marine algae. J. Hazard. Mater. 92(3): 253-262.

Kozdra Z, Van Elsas JD (2001). Structural diversity of microbial communities in arable soils of a heavily industrialized area determined by PCR-DGGE fingerprinting and FAME profiling. Appl. Soil Ecol. (17): 31-42.

Lopez A, Lazaro N, Priego JM, Marques AM (2000). Effect of pH on the biosorption of nickel and other heavy metals by Pseudomonas fluorescens 4F39. J. Ind. Microbiol. Biotechnol. (24):146-151.

Macciotta LE, Creamer NJ, Essa AM, Brown NL (2007). A new approach for the recovery of precious metals from solution and from leachate derived from electronic scrap. Biotechnol. Bioeng. 96: 631-639.

Mameri N, Boudries N, Addour L, Belhocine D, Lounici H, Grib H, Pauss A (1999). Batch zinc biosorption by a bacterial nonliving Streptomyces rimosus biomass. Water Res. (33): 1347-1354.

Manniatis T, Fritsch EF, Sambrook J (1982). Molecular cloning: A laboratory manual. 3rd. ed, Cold Spring Harbor laboratory, Cold Spring Harbor, NV.

Mueller JH, Hinton J (1941). A protein-free medium for primary isolation of the Gonococcus and Meningococcus. Proc. Soc. Exp. Biol. Med. (48):330-333.

Noghabi KA, Zahir HS, Yoon SC (2007). The production of a cold-induced extracellular biopolymer by Pseudomonas fluorescens BM07 under various growth conditions and its role in heavy metals absorption Process. Process Biochem. 42 (5): 847-855.

Ozdemir G, Ozturk T, Ceyhan N, Isler R, Cosar T (2003). Heavy metal biosorption by biomass of Ochrobactrum anthropi producing exopolysaccharide in activated sludge. Biores. Technol. 90 (1): 71-74.

Pardo R, Herguedas M, Barrado E, Vega M (2003). Biosorption of cadmium, copper, lead and zinc by inactive biomass Pseudomonas putida. Anal. Bioanal. Chem. 37(1):26-32.

Parry J (2009). Metal smelting plants poison hundreds of Chinese children. BMJ (339):3433.

Prescott LM, Harley JP, Klein DA (2002). Microbiology, Fifth Edition. N.Y., US. McGraw-Hill Higher Education. pp. 95-112.

Purakir PJ, Pakniker KM (1999). Biosorption of lead, cadmium and zinc by Pseudomonas fluorescens. Environ. Biochem. Biophys. 45: 161-165.

Raval J, Diruggiero J, Robb FT, Hill RT (2000). Cloning and sequence analysis of the mercury resistance operon of Streptomyces sp. Strain CHR28 reveals a novel putative second regulatory gene. J. Bacteriol. (182): 2345-2349.

Remacle J (1990). The cell wall and metal binding. In: Biosorption of heavy metals. B. Volovsky (ed.) CRC Press, Boca Raton, USA, Florida, pp. 83-92.

Roane TM (1999). Lead resistance in two bacterial isolates from heavy metal-contaminated soils. Microb. Ecol. (37): 218-224.

Rudzinska C, Herzig-Marx R, Lin J, Szpiro A, Johnson B (2004). Pathogen detection using headspace analysis. Proceedings of the Scientific Conference on Chemical and Biological Defense Research. MIT Lincoln Laboratory, 244 Wood Street, Lexington, MA 02420. Hunt Valley, Maryland.

Sağ Y, Kutsal T (2000). Determination of the biosorption heats of heavy metal ions on Zoogloea ramigera and Rhizopus arrhizus. Biochem. Eng. J. (6): 145-151.

Sanger F, Nicklen S, Coulson AR (1977). DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. 74(12): 5463-5467.

Silva RMP, Rodríguez AA, De Oca JM, Moreno DC (2009). Biosorption of chromium, copper, manganese and zinc by Pseudomonas aeruginosa AT18 isolated from a site contaminated with petroleum. Biotechnol. 100(4): 15-33.

Silver S (1996). Bacterial resistances to toxic metal ions - a review Gene 179 (1): 9-19.

Tenover FC, McGowan JJ (1996). Reasons for the emergence of antibiotic resistance. Am. J. Med. Sci. 311(9): 9-16.

Tobin JM, Copper DG, Neufeld R (1984). Uptake of metal ions by Rhizopus arrhizus biomass. Appl. J. Environ. Microbiol. (47):821-824.

Uslu G, Tanyol M (2006). Equilibrium and thermodynamic parameters of biosorption by biomass of Pseudomonas putida. Anal. Bioanal. Chem. 37(1):26-32.

Valls M, Delorenzo V (2002). Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution. FEMS Microbiol. Rev. (26) 327.

Vijayaraghavan K, Yun YS (2007). Utilization of fermentation waste (Corynebacterium glutamicum) for biosorption of reactive black 5.
from aqueous solution. J. Hazard. Mater. (141): 45–52.
Volesky B, Holan ZR (1995). Biosorption of heavy metals. Biotechnol. Prog. 11: 235-250.
Volesky B, May-Phillips HA (1995). Biosorption of heavy metals by Saccharomyces cerevisiae. J. Appl. Microbiol. Biotechnol. 42:797-806.
Wang J, Chen C (2006). Biosorption of heavy metals by Saccharomyces cerevisiae: A review. Biotechnol. Adv. (24): 427-451.
Wasi S, Jeelani G, Ahma M (2008). Biochemical characterization of a multiple heavy metal, pesticides and phenol resistant Pseudomonas fluorescens strain. Chemosphere 71(7): 1348-1355.
Zolgharnein H, Azmi MLM, Saad MZ, Mutalib AR, Mohamed CAR (2007). Detection of plasmids in heavy metal resistance bacteria isolated from the Persian Gulf and enclosed industrial areas. Iran. J. Biotechnol. (5): 232-239.
Zouboulis AI, Loukidou MX, Matis KA (2003). Biosorption of toxic metals from aqueous solutions by bacteria strains isolated from metalpolluted soils. Process Biochem. 39(8): 909-916.