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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 (Volesky 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 removial (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: $CdCl_2$-$2H_2O$, $CuSO_4$-$5H_2O$, $CoCl_2$, $ZnSO_4$-$7H_2O$ and $Pb(NO_3)_2$ 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: $CdCl_2$-$2H_2O$, $CuSO_4$-$5H_2O$, $CoCl_2$, $ZnSO_4$-$7H_2O$, and $Pb(NO_3)_2$. 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: Pb^{2+}> Zn^{2+}> Co^{2+}> Cd^{2+}> Cu^{2+}) were selected for characterization, identification. Before and after the treatment with $Pb(NO_3)_2$, 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 $CdCl_2$-$2H_2O$, $CuSO_4$-$5H_2O$, $CoCl_2$, $ZnSO_4$-$7H_2O$, and $Pb(NO_3)_2$ 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 HNO_3 with Pb $(NO_3)_2$. 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 biomasses 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 $mg\text{-}l^{-1}$ 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) $=V (C_I - C_F) / w$

Where, C_I = initial metal concentration (mg/L), C_F = 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 $CdCl_2$ and

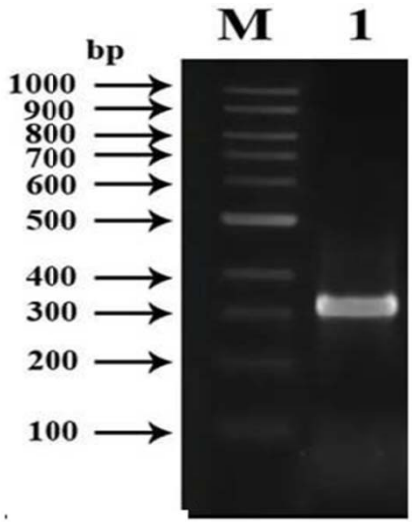

Figure 1a. Agarose gel electrophoresis for PCR products of 16S-rRNA gene analysis M: 1 kbp DNA marker, 1: PCR product of *B. anthracis* PS2010.

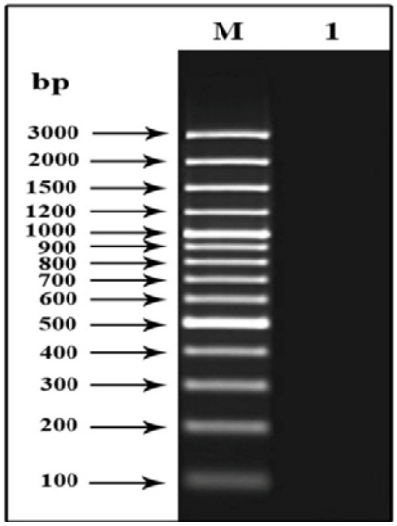

Figure 1b. Agarose gel electrophoresis of plasmid profile for *B. anthracis* PS2010. M: DNA marker, 1: Plasmid of *B. anthracis* PS2010.

was found to be highly tolerant to $ZnSO_4$-$7H_2O$. 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 16Sr-RNA gene partial sequence of isolated strain revealed close similarity with *B. anthracis* TC-3, *B. anthracis* A0248, *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 characterristic 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 avirulant. 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 avirulant 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^{2+}, Cd^{2+}, Cr^{2+}, 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^{2+}, Cr^{2+}, 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+}

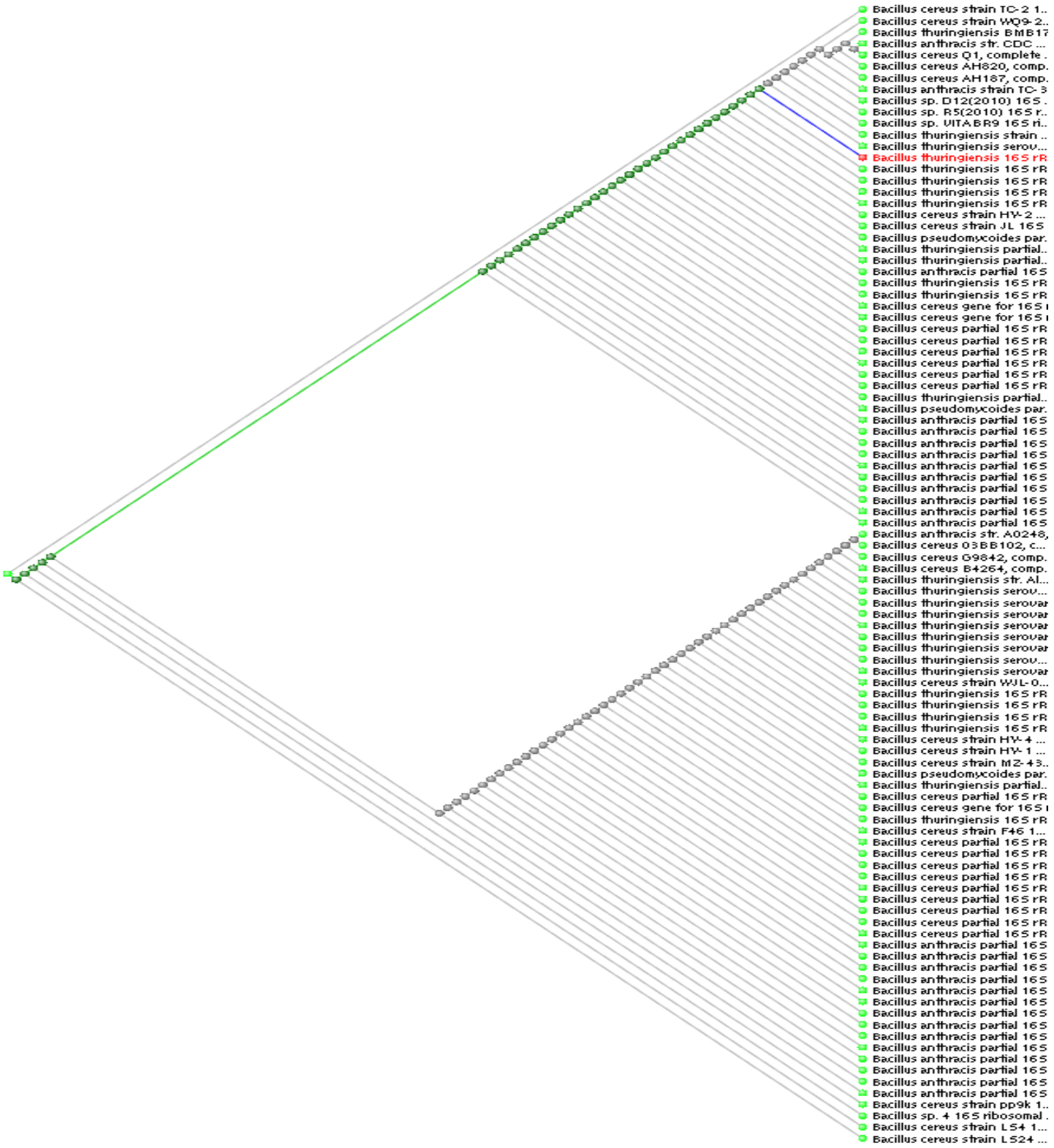

Figure 2. Phylogenetic tree based on 16S-rRNA gene partial sequences obtained from the respective band matched with the National Center For Biotechnology Information (NCBI) nucleotide sequence database (GenBank).

and Pb^{2+} (%) decreased by increasing temperature. For Cd^{2+}, Cu^{2+} and Co^{2+} 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-

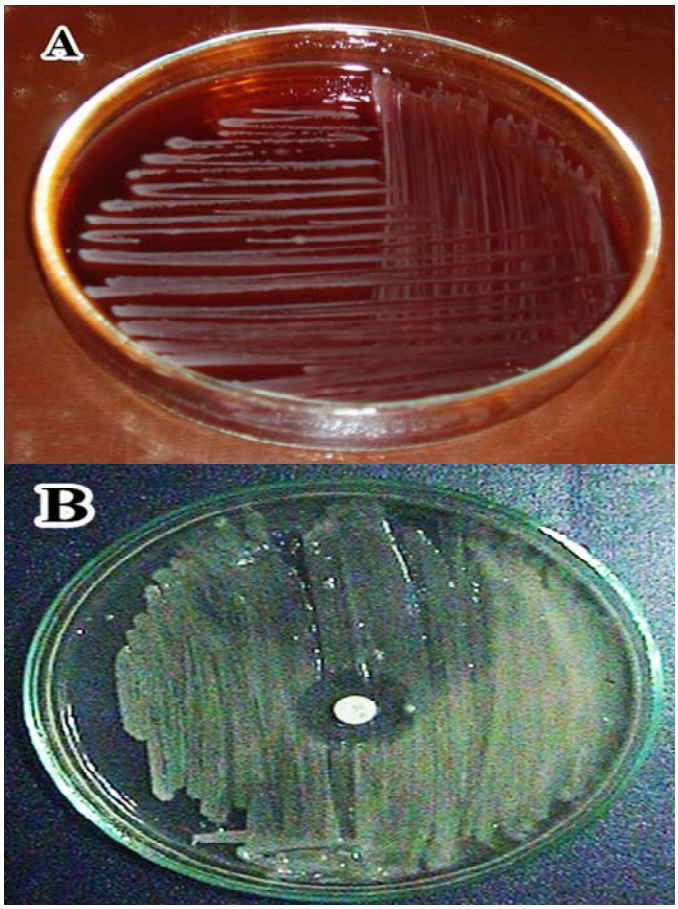

Figure 3. Blood agar culture showing grayish-white growth with non-hemolytic activity (A) and penicillin sensitivity test on Mueller-Hinton agar (B) of *B. anthracis* PS2010.

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 obtained by Ozdemir et al. (2003). On the other hand, pH 5 was optimum for Zn^{2+} and Pb^{2+} uptake by *B. anthracis* PS2010. In the case of Cd^{2+} and Co^{2+}, increasing the alkalinity of the solutions was followed by large decrease in the uptake of these metals especially Co^{2+}. By increasing the pH over 5 in the case of Zn^{2+} and Pb^{2+}, the uptake started to decrease gradually (Ozdemir et al., 2003). In the case of Cu^{2+}, the uptake seemed to be slightly increased by increasing the pH from 5 to 7 (Figure 5). Silva et al. (2009) revealed the chromium level sorbed by *P. aeruginosa* AT18 biomass with 100% removal was in pH range 7-7.2. At low pH, the Cd^{2+} accumulation was decreased and caused increased competition between hydrogen and cadmium ions for binding sites on the cell surface or by an increase in metal efflux pump activity due to an increase in the proton gradient that drives the efflux pump. The pH 7 was optimum for Pb^{2+} and Co^{2+} accumulation by *B. anthracis* PS2010, while pH 5 was optimum for Zn^{2+} uptake. It was reported that pH 4.5 was optimum for biosorption of lead by *Citrobacter* strain MCM B-181 and pH < 3 or > 5 resulted in lower biosorption efficiency of lead (Puranik and Pakniker, 1999; Guangyu and Thiruvenkatachari, 2003). At highly acidic pH (<3) lead ions compete with hydrogen ions on the binding sites of microbial cells. However, at higher pH (>5) solubility of lead was lowered (Chang et al., 1997). The variation in external pH can also affect the degree of protonation of potential ligand that contribute to metal binding (Tobin et al., 1984).

The contact time between the bacterial cells and the metal solutions is an important factor affecting the metal uptake. Figure 6 shows the maximum uptake for Cd^{2+}, Cu^{2+} and Zn^{2+} occurred after 24 h. However, 18 and 48 h was optimum for Co^{2+} and Pb^{2+} uptake. These results agree with that of Remacle (1990). The uptake of Co^{2+} decreesed 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*as 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 $Pb(NO_3)_2$, 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 $Pb(NO_3)_2$-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.

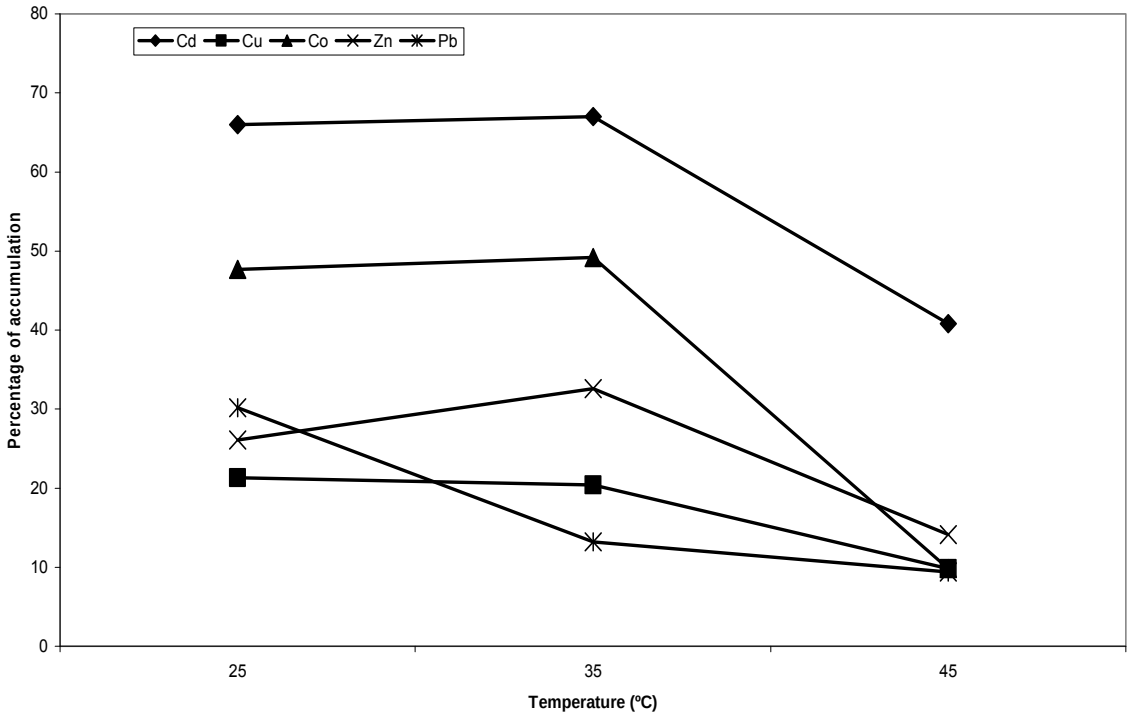

Figure 4. Effect of different temperatures on heavy metal accumulation by *B. anthracis* PS2010.

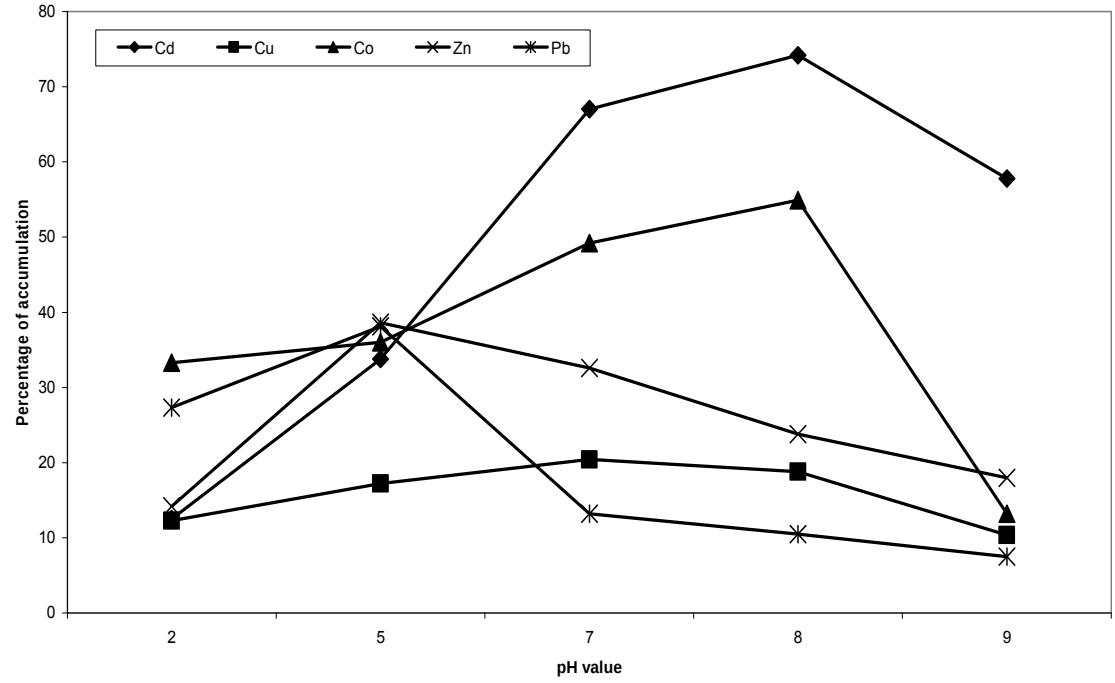

Figure 5. Effect of different pH values on heavy metal accumulation by *B. anthracis* PS2010.

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 nano-particles. 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

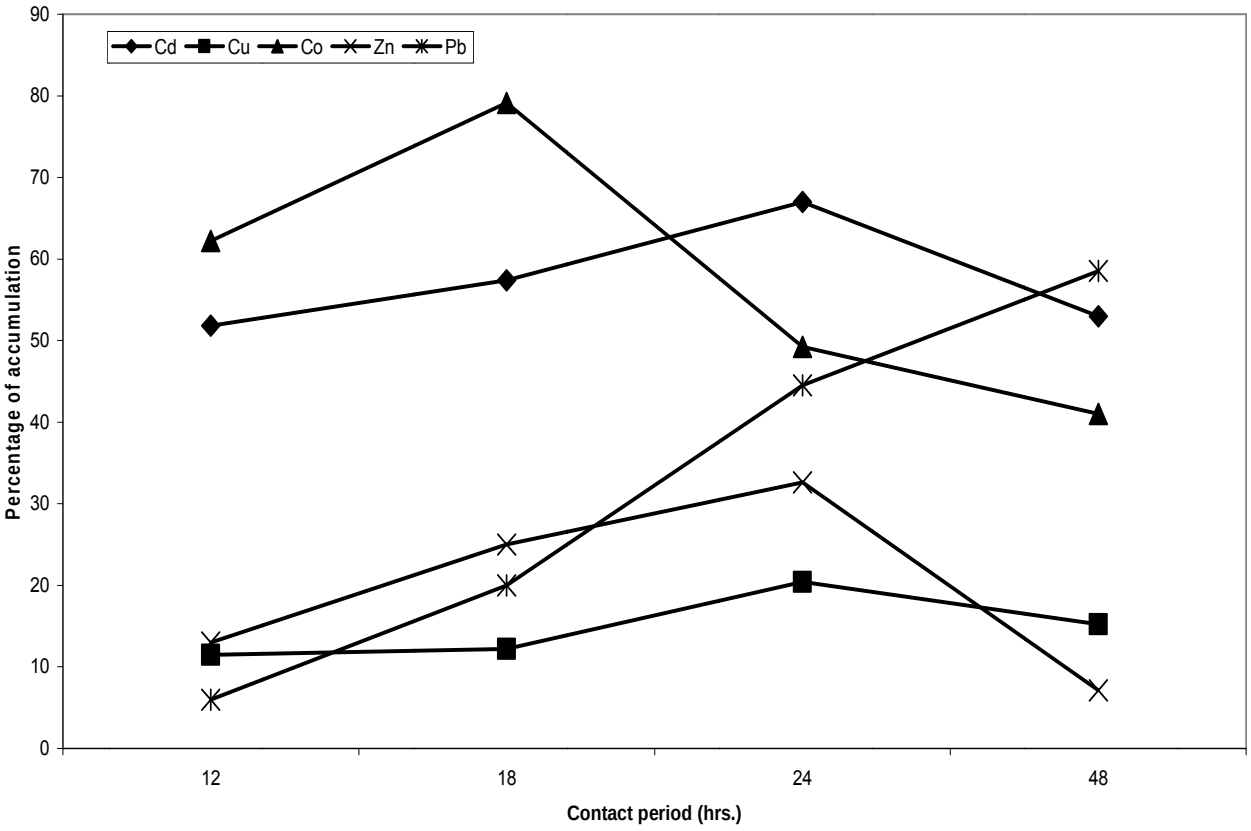

Figure 6. Effect of different contact periods on heavy metal accumulation by *B. anthracis* PS2010.

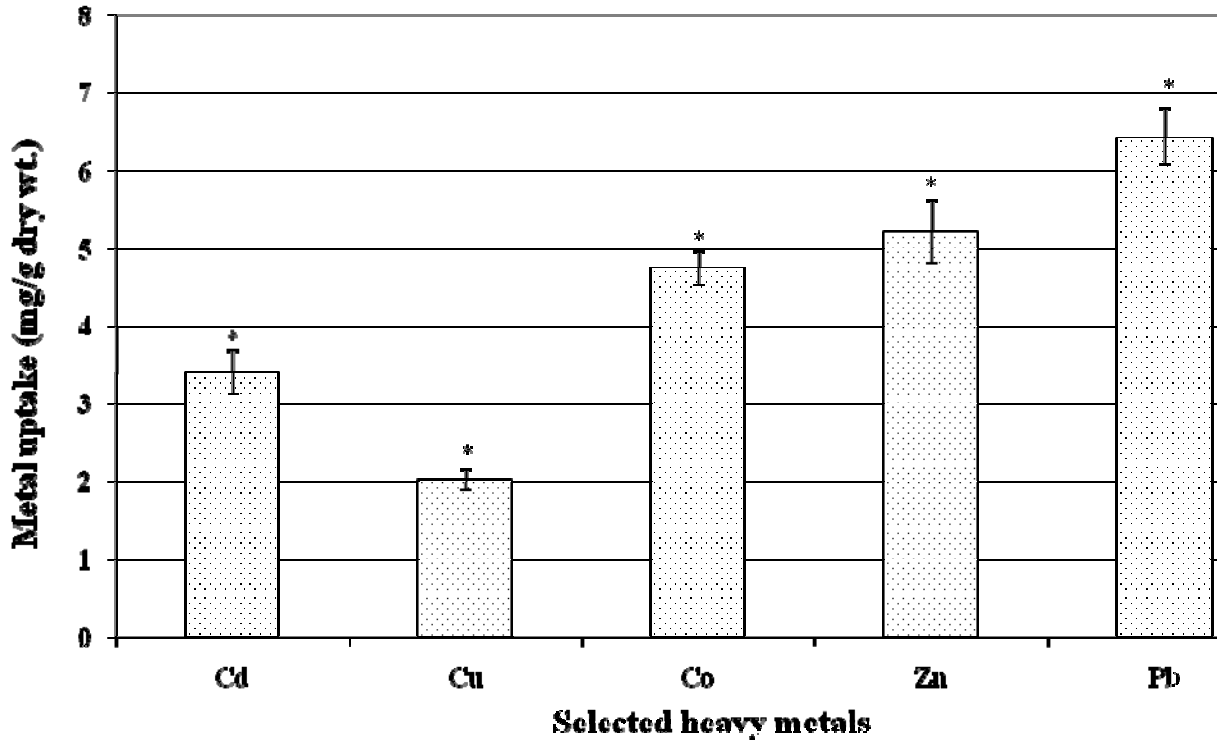

Figure 7. Metals uptake by *B. anthracis* PS2010 under the optimum conditions.

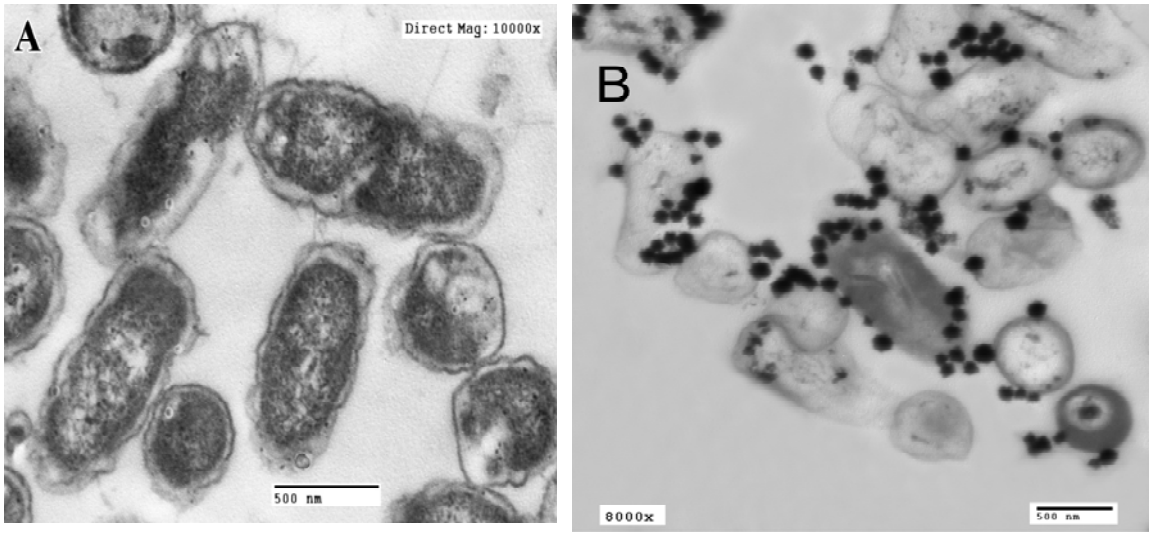

Figure 8. Transmission electron micrographs of *B. anthracis* PS2010, A: untreated cell; B: showing extracellular accumulation of Pb nanoparticles.

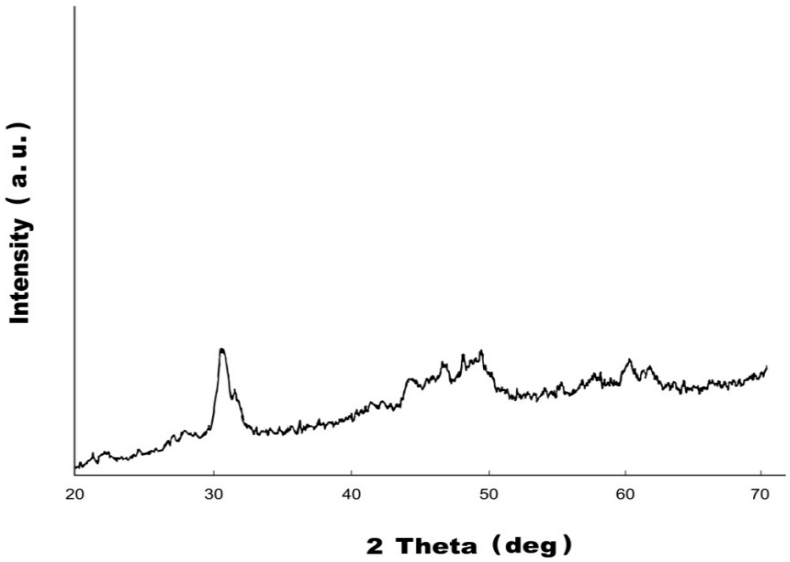

Figure 9. X-ray diffractogram of PbO nanoparticles synthesized by *B. anthracis* PS2010.

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).

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