

Biosorption and Bioaccumulation of Some Heavy Metals by *Deinococcus Radiodurans* Isolated from Soil in Basra Governorate- Iraq

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Abstract

The bacterium *Deinococcus radiodurans* has been isolated from soil. On the basis of morphological, biochemical, 16S rRNA gene sequencing and phylogeny analysis revealed that, the isolates were authentically identified as *D. radiodurans*.

D. radiodurans showed significant resistance to high concentrations of Pb and Cd, but it was more tolerant to Cd than Pb. Minimum inhibitory concentration was 400 mg l⁻¹ for Pb, while it was 600 mg l⁻¹ for Cd. The potent bacterium has the optimal bioaccumulation capacity differ according to metal type, concentration, and contact time. In bioaccumulation experiment, the results showed the highest increase in accumulation of Pb in the concentration 50 mg l⁻¹ at 6 h of incubation (0.33 mg g⁻¹), while the lowest accumulation was in concentration 5 mg l⁻¹ (0.029 mg g⁻¹) at 2h of incubation. For Cd the results showed maximum accumulation at 24h for concentration 100 mg l⁻¹ then decreased at 48 h.

The results of biosorption experiment showed that *D. radiodurans* has a good ability to absorption both Pb and Cd in considering to the metals concentrations and times. This which can be clarified from the elevated percentage of Pb absorption (63.46%) in concentration 50 mg l⁻¹ and during 2h. For biosorption of Cd the was decreased with the increasing time and the high biosorption noticed during 2h at concentration 50 mg l⁻¹ (31.23%).

Keywords: *Deinococcus radiodurans* ; Heavy metals; Minimum inhibitory concentration; Bioaccumulation; Biosorption

Introduction

D. radiodurans is one of the most known radiation resistant organisms. It can live in cold, dehydration, vacuum and acid, and is therefore known as a polyextremophile. It is found in habitats rich in organic materials, such as soil, feces, meat, or sewage, in addition to that, it's isolated from dried foods, room dust, medical instruments and textiles [1].

The manufacturing of energy from nuclear power factories, uranium mining, nuclear weapons production and nuclear accidents are the major cause of release of radionuclide's into the environment. The nuclear wastes typically contain inorganic and organic contaminants that include radionuclide's, heavy metals, acids/base and solvents. The high radiation level, in combination with the chemical hazards cause intensive damage to ecosystem and living organisms. The clean -up of nuclear waste by physiochemical methods is impractical and the cost is prohibitive. As a result a less costly in situ bioremediation technology is being investigated as a potential substitute method for treating such contaminated sites. The development of bioremediation strategies using *Deinococcus* spp, is therefore vital for the clean up of contaminated sites with radioactive waste. As these sites rarely contaminated by a single chemical, it is necessary to developed strain to be multi- resistant to various toxic agents [2].

Bioremediation is a natural process which depends on bacteria, fungi, and plants to altering pollutants as these organisms perform their normal life functions. These organisms have the ability of exploiting chemical contaminants as an energy source in their metabolic processes. Therefore, bioremediation affords alternative tool to destroy or reduce the risky contaminants through biological activity and this method has an effective cost [3].

Among the different methods, bioaccumulation and biosorption

are potential for the removal of metals [4]. The active mode of metal accumulation of living cells is in most cases referred to bioaccumulation which relies on intrinsic biochemical and structural properties, physiological and genetic adaptation, environmental modification of metal specification, availability and toxicity [5]. Bioaccumulation is defined as the uptake of toxicant by living cells and transports it into the cell [6] and is a growth dependent process mediated only by living biomass [7]. The process of biosorption is possible by both living and dead biomasses [8]. The ability of certain species of bacteria in accumulating heavy metals was investigated inclusively [9,10]. Hence the natural organisms, either indigenous or extraneous can be used for bioremediation of heavy metals [11].

The present study aimed to isolate *Deinococcus radiodurans* from soil and identifying it biochemically and molecularly, in addition to determining their ability to remediation Pb and Cd through bioaccumulation and biosorption processes.

Materials and methods

Isolation of bacteria

Three soil samples (30 gm each) were collected from Um- Qasr

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district, south of Basra city- Iraq during the January (2013). The samples were collected using a sterile plastic bag and transferred within 2 h to laboratory for analysis. For isolation of *Deinococcus radiodurans*, one gram of air dried soil sample was serially diluted using distilled water and spread over Tryptone Glucose Yeast extract (TGY) medium (0.5% tryptone, 0.1% glucose and 0.3% yeast extract) agar solid plates. The plates were incubated at 30°C for 24 h.

Bacterial characterization

Properties of the isolates that included gram reaction, citrate utilization, indole production, nitrate reduction, catalase, fermentation of D- glucose, arginine, lactose and mannose, hydrolysis of casien, and gelatin liquefaction tests were determined according to Murray [12].

16S rRNA based identification

Bacterial samples were identified by sequencing of the 16S rRNA gene. To determine the identity of bacterial samples, the amplified 16S rRNA gene PCR products obtained from total genomic DNA using primer set 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'), [13] were sequenced commercially. DNA sequences obtained were compared to sequences available online in the Gene Bank database (www.ncbi.nlm.nih.gov). Homology search was performed using Bioinformatics tools available online, BLASTn www.ncbi.nlm.nih.gov/BLA [14].

Determination of minimum inhibitory concentrations (MIC) for Cd and Pb The MIC of Cd and Pb of bacteria were determined by disc diffusion method [15]. The concentrations of Cd and Pb were between 100 to 1000 mg^l⁻¹. Filter paper disks were saturated with heavy metals for 30 min, and then added to nutrient agar plates which incubated for 24h at 30°C. The salts of CdCl₂ and Pb (NO₃)₂ were used to prepare mother solution of these metals in sterile distilled water.

Bioaccumulation of heavy metals by Bacteria

Bacteria were grown in TGY broth containing different concentrations of lead (5, 10, 25, 50) mg^l⁻¹ and for cadmium 10, 20, 50, 100 mg^l⁻¹ for 2, 4, 6, 24 and 48 h and incubated at 30°C in a shaker incubator at 180 rpm. Two replicates for each concentration have been done plus, one as a control. The bacterial cells were harvested by centrifugation at 6000 rpm for 15 min and suspended in 1 ml of distilled water, oven - dried and weighed. The dried biomass were then digested as follow: 100 ml beaker containing the dried cells, 5 ml concentrated nitric acid was added; the beaker was placed on a hot plate, stirred continuously, and heated initially at a medium rate for 5 min. Then, the beaker was heated on maximum setting until nitrogen oxide fumes were given off for a short time and a white residue was left. The beaker was left to cool for about 2 min and digestion was repeated with an additional 2 ml of concentrated nitric acid; this time it was heated until brown nitrogen oxide fumes almost ceased to appear. The beaker was cooled again for about 2 min and then 2 ml of 1:1 hydrochloric acid (35- 37%) was added. The mixture was heated at a medium rate for 3 min. After that it was cooled to room temperature and made up to 25 ml or bigger volume with distilled water.

Metal concentrations were measured by an atomic absorption spectrophotometer (Thermo Scientific ICE 3000 Series AA Spectrometer, USA) [16]. The metals accumulation then calculated using this equation:

$$E. Con. = A*B/D$$

Where E. Con = the concentration of heavy metals in (mg/g)

A= Concentration of heavy metals from calibration curve

B= final concentration of sample (mg/l)

D= dry weight of sample (gram)

Biosorption experiment

The equilibrium, kinetics data of the biosorbent *D. radiodurans* were obtained by performing batch experiments. The experiments were carried out in 250 ml flasks to which 100 ml of single ion metals solution of Cd and Pb, and 1 ml of biomass (exponential phase) was added. The mixture was stirred at 180 rpm at 30°C and 15 ml of sample was collected at interval times (2, 4, 6, 24, and 48 h) centrifuged at 6000 rpm for 15 min in a centrifuge. The remaining concentration of metals was analyzed by the atomic absorbance spectrophotometer. An experiment was carried out twice and the mean values were reported. The difference between the initial metal ion concentration and the final metal ion concentration was considered as metal bound to the biosorbent [17].

Statistical analysis

The data obtained on the Bioaccumulation and biosorption of different metals at different concentration and time interval, by the bacterium *D. radiodurans* subjected to Statistical analysis using the SPSS program (SPSS Inc., Chicago, IL Version 15.0). The data were analyzed through analysis of variance (ANOVA). To detect the statistical significance of differences (P<0.05) between means.

Results and Discussion

Characterization and molecular identification of isolated bacteria

The selected bacterium was characterized and identified by using morphological, physiological and biochemical tests (Table 1). Bacteria were presumptively identified as *Deinococcus* sp. According to Chaturvedi [18]. Also the bacteria were subjected to 16S rRNA gene sequence analysis. The sequence of 16S rRNA of bacteria was submitted to Blastn (database 16S ribosomal RNA sequences (Bacteria and

Tests	Characteristics observed
Morphology	
shape	Cocci
pigment	pink
Gram reaction	+
Biochemical reaction	
Oxidase test	+
Catalase test	+
Indol formation	+
Citrate utilization	+
Gelatin liquefaction	+
Nitrate reduction	-
Fermentation	
D-glucose	+
Arginine	+
Lactose	-
Mannose	+
Casien	+
"+"and "-" indicate positive and negative reactions, respectively	

Table 1: Morphological and biochemical characteristics of *D. radiodurans*.

Archaea); Megablast) (www.ncbi.nlm.nih.gov/blast). That indicated a close genetic relatedness of bacteria with the rRNA sequence of *D. radiodurans*.

Minimum inhibitory concentration

The MIC is the lowest concentration of the heavy metals that completely inhibited bacterial growth [19]. *D. radiodurans* showed significant resistance to high concentrations of Pb and Cd, but it was more tolerant to Cd than Pb. MIC was 400 mg l⁻¹ for Pb, while it was 600 mg l⁻¹ for Cd. Chaturvedi [18] reported different results in his study, he reported that the different isolated of *Deinococcus* (GrK2, Grk4, GrK5 and DR1) were sensitive to Cd⁺², but they exhibited varying levels of tolerance. Grk2 was being extremely sensitive to Cd⁺², DR1 is moderately tolerant, while Grk4 and Grk5 showed comparable tolerance, and also he mentioned that the toxicity of Cd affected by growth state, with stationary phase cells being more sensitive than the exponential phase. Hua et al. [20] reported that *D. radiodurans* R1 does not exhibit strong resistance to heavy metal Hg (II), Ag (I), Cr (VI) and Pb (IV), and recorded MIC (300 , 1600 300 and 3200 μM) for these metals respectively.

From the results of the present study there is a difference in results recorded in comparison with other studies results recorded previously, and this can be explained as the isolated strains was different and the growing conditions such as growth stage also different. In addition to the high concentration of heavy metals in the study soil, which enhance the ability of tolerant to these bacteria. Those can be supported by the result of Chaturvedi [18], who reported that, the growth phase depending differences in tolerance. Qi and Hulett [21] reported that, the tolerance of growing *B. thuringiensis* DM55 cells has been shown to vary at different growth stages, supporting the existing evidence that the structural features of gram positive bacterial cell walls are affected by the developmental state of the cell. Another reason for these results differences is the variance in design of experiment, such as growth media composition as clarified by Kumar et al. [22]. Complex media such as LB precipitate Pb as a result of sequestration with organic moieties thus reduce the bioavailability of Pb to the bacterial strains [23].

Bioaccumulation study

From the results shown in Figure 1 there is an increasing in the accumulation of Pb with the increasing of the concentration. The highest accumulation occurs in the concentration 50 mg l⁻¹ after 6h of incubation was 0.33 ± 0.0007 mg g⁻¹, while the lowest was 0.029 ± 0.268 mg g⁻¹ after 2h. In addition to that, the rising of incubation period may reduce the accumulation for all concentrations.

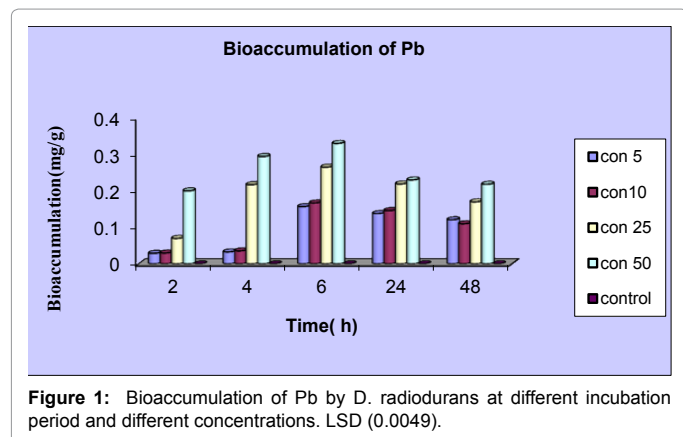


Figure 1: Bioaccumulation of Pb by *D. radiodurans* at different incubation period and different concentrations. LSD (0.0049).

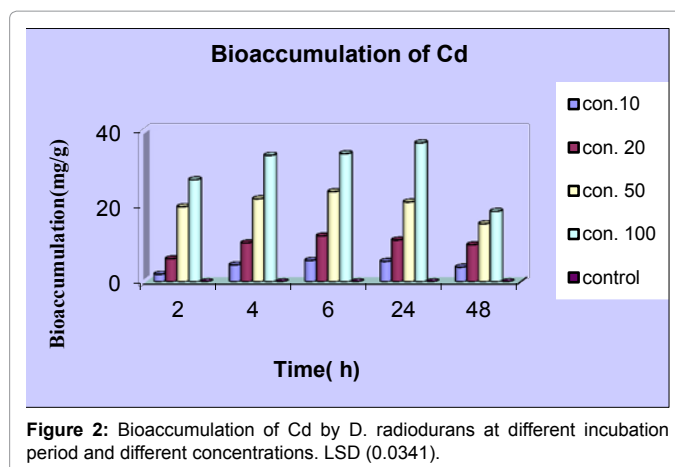


Figure 2: Bioaccumulation of Cd by *D. radiodurans* at different incubation period and different concentrations. LSD (0.0341).

The accumulation of Cd increase in parallel with the increasing of concentration for all studying time (Figure 2). The figure clarifies increasing in Cd accumulation with the times (2, 4 and 6 h) for concentrations 10, 20 and 50 mg l⁻¹, then decrease during 24 and 48h. For concentration 100 mg l⁻¹, accumulation increase within 2, 4, 6, and 24h, then decrease with 48h. The analysis of variance of bioaccumulation of Pb and Cd between time and concentration was significant (P<0.05) in all treatments as show by LSD value.

The results of Pb and Cd accumulation showed that, this bacterium was able to accumulate high amounts of Cd 36.86 ± 0.007 mg g⁻¹ in comparison to 0.33 ± 0.0007 mg g⁻¹ for Pb. This may be due to difference in toxicity of these metals to this bacterium and to its intrinsic properties which help it in occupying varying tolerance, also the history of pollution of the soils from which bacterium isolated by these metals play critical tool in development ability of these bacteria to deal with such contaminations, this which agrees with the results of the present study where it show high MIC value for Cd than this for Pb for this bacterium.

From the results the accumulation increase with time, then start decreasing over specific time. This agrees with Ray et al. [24] who reported that, the accumulation of Pb by *B. cereus* increased with time, then starts decreasing. It may be concluded that, metal binding sites became saturated after specific time. The maximum accumulation of both of metals by this bacterium occurs in the highest concentration of metals then start decreasing with increasing of the time, this observed by Tunali et al. [25] who reported that, the amount of metal ions accumulated per unit mass of *Bacillus* sp. (ATS-1) increased first with increasing of the initial metal ion concentration and reached to a saturation value. Then the value changes with the initial metal ion concentration change. Also Ozdemir et al. [26] reported that, the accumulation of chromium (VI), cadmium (II) and copper (II) by *Pantoea* sp. TEM18 increase with high initial metals concentration, then reach to saturate so the accumulation decrease. Table 2 shows a comparison between different microorganisms and *D. radiodurans* in the present study.

Biosorption study

The results of the present study (Table 2) showed that *D. radiodurans* has a good ability to absorption Pb and Cd. The highest percentage of Pb absorption was 63.46% in concentration 50 mg l⁻¹ for 2h. Biosorption of Pb increased with the increase of time for concentration 5, 10 and 25 mg l⁻¹, then start decreasing with increase time for concentration 50

Biomass	Heavy Metals	Bioremediation capacity (mgg ⁻¹) or %	Heavy Metals	Reference
<i>Saccorhiza polyschides</i>	Cd	95	Cd	Loderio et al. [32]
<i>Enterobacter sp.</i>	Pb	50	Pb	Lue et al. [33]
<i>Bacillus cereus</i>	Pb	36.71	Pb	Babak et al. [34]
<i>Micrococcus sp.</i>	Zn	84.27	Zn	Hussein et al. [35]
<i>Aspergillus niger</i>	Cr	133	Cr	Goyal et al. [36]
<i>D. radiodurans</i>	Cd	36.86	Cd	Present study
	Pb	0.33	Pb	
<i>D. radiodurans</i>	Cd	31.23%	Cd	Present study
	Pb	63.46%	Pb	

Table 2: Comparison of different microorganisms based on their maximum capacity for bioremediation of different heavy metals in comparison to *D. radiodurans* in the present study.

Concentration (mg/l)	% Biosorption of Pb at different times (h.)				
	2	4	6	24	48
5	22.72±0.007	22.85±0.141	23.50±0.007	27.91±0.007	30.43±0.014
10	28.15±0.007	30.32±0.021	30.40±0.014	30.43±0.007	30.45±0.007
25	41.8±0.007	43.20±0.141	44.00±0.141	44.20±0.070	44.33±0
50	63.46±0.007	59.97±0.007	39.33±0.007	33.40±0	31.89±0.070
LSD= 0.001					
Concentration (mg/l)	% Biosorption of Cd at different times (h)				
	2	4	6	24	48
5	18.97±0.014	18.57±0.014	17.65±0.014	16.10±0.707	15.58±0.007
10	22.80±0.070	22.76±0.007	21.00±0	20.71±0.007	20.14±0.007
25	27.64±0.014	27.60±0.070	27.58±0.021	26.57±0.014	26.52±0.014
50	31.23±0.014	31.12±0.014	30.75±0.021	30.64±0.014	30.32±0.014
LSD= 0.024					

Table 3: Biosorption (%) of Lead and Cadmium at different period of incubations and different concentrations by *D. radiodurans*.

mg⁻¹. Explanations for these results is that at first three concentrations, the increase in biosorption with the time due to the sorbent sites were not saturated, but in concentration 50 mg⁻¹, the concentration was sufficient to saturate these sites, so the maximum absorption occurs in the first two hours. Tarangini [27], reported that, biosorption of arsenic by mixed cultures of *Pseudomonas aeruginosa* and *Bacillus subtilis* was increased with the increase time.

In related to the effect of Pb concentration, results showed sorption increase with the increasing Pb concentration. Taty-Cortodes et al. [28], showed that, the initial ion concentration exhibits quite an interesting effect on the equilibrium sorption capacity of the *Pinus sylvestris* for Cd (II) and Pb (II). At a fixed biosorbent dose, pH and temperature, the equilibrium sorption capacity, improved with higher initial ion concentration. The ion removal was highest concentrated dependent. The increase in the biosorbents loading capacity as a function of metal ion concentration was believed to be due to a high driving force for mass transfer.

The results of the present study of Cd sorption showed decrease of biosorption with increasing time (Table 2). The high sorption noticed after 2h at concentration 50 mg⁻¹. This is similar to Anzeze et al. [29], who reported that, the rate of adsorption of Cd by *Eichhornia*

crasippes was very fast at first and over 95 % of total biosorption of Cd (II) ions occurs in the first 5 minutes and thereafter it proceeds at a slower rate and finally no further significant adsorption is noted beyond 20 minutes of contact time. For effect of metal concentration on biosorption, results show increases in Cd sorption with increasing metal concentration. The initial concentration provides an important driving force to overcome all mass transfer resistance of metal between the aqueous and solid phases [30]. The increasing amount of metal adsorbed by the biomass will be increased with initial concentration of metals. Optimum percentage of metal removal can be taken at high initial metal concentration. Thus, at a given concentration of biomass, the metal uptake increases with increase in initial concentration [31].

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