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Bio removal of Cadmium element by use Bacillus cereus

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ABSTRACT

samples were collected from different agricultural soils, the bacterial isolates that isolated from these samples, were Bacillus cereus, which were identified based on phenotypic characteristics and molecular diagnosis. The bioremoval process was carried out using Bacillus cereus and cadmium at concentrations (0.5, 0.2, 0.1 mg/ml) at a temperature of 37 ° C and pH 7 for a period (24,48,72 hours). the results showed that The highest rate of cadmium removal was 86% at a concentration of 0.1 mg / ml and the lowest rate of removal was 34% at a concentration of 0.5 mg / ml. Also ,it has been study the effect of the incubation period on removing heavy element ions, and it was found that the best period was 24 hours compared to 48 and 72 hours . and showed that the highest removal ratio 86 % .

1. Introduction

Heavy elements have a density higher than 5 g / cm3, which is more than five times the density of water **(Duffus ,2002)**. The toxic elements are present permanently and at basic levels of natural origin because it comes from the weathering processes of the mother rocks **(Facchinell *et al.*, 2001)** emitted from the sources humanity includes oil refineries, leather and fertilizer

industries, textile tanning, batteries, paint products, car fuel and household waste. It plays a major role in increasing toxic elements in the watery ocean **(Al-Shammari, 2013)** and humans and their health and all living organisms, and the danger of these elements is that they are permanent and persistent in the environment **(Kim *et al.*, 2007).**The health risks of heavy metals depend on the level and duration of exposure

with Acute exposure : Exposure to large amounts of heavy metals in a short period of time

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and Chronic exposure: exposure to small amounts of heavy metals over a long period of time (**Young, 2000)**. Cadmium is considered a heavy and highly toxic element. Symptoms of poisoning may occur after years of accumulation in the body. The symptoms of poisoning include disturbance of kidney function, osteomalacia, high blood pressure and an enlarged heart. **(*Toman et al . , 2005*)** .Effective microbiology technology has been widely used in many countries of the world to improve the environment, including biological treatment plants **(Hega and Parr, 1994**) ,These biological communities will play an important role in preserving the biosphere because of these desirable advantages that distinguish these bacteria from other microorganisms. Academic and industrial interest focuses initially on soil bacteria **(Saha and santra, 2014)** due to their diffusion, size and ability to grow under controlled conditions. On them and their resilience for a wide range of environmental conditions **(Srivastava et al, 2015).**

So, the aim of this study is to isolate and diagnosis Bacillus cereus from agricultural soil and detect its ability to remove the cadmium element.

2. Materials and methods

2.1 Collect samples

Soil samples were collected from agricultural fields with three replications. The samples were placed in clean and sterile bags and transported to the laboratory.

2.2 Isolation

One gram of soil sample was serially diluted in sterile distilled water to obtain a concentration ranging from 10^{-1} -10^{-3} . Samples were placed in a water bath at 60 ° C for 60 minutes , a volume of 0.1 ml of each diluent was transferred aseptically to plates of nutrient agar and incubated at 37 ° C for 24 hours. After the onset of growth, individual colonies were isolated separately **(Aslim et al., 2002**).

2.3 Identification based on 16SrRNA gene

16S rRNA gene was detected by DNA extraction from bacterial isolates, prefixes F (GTGAGGTAACGGCTCACCAA) were used R (CTTCAGCACTAAAGGGCGGA) that manufactured by (Bioneer, South Korea), and after the completion of the PCR process, the products were sent to Macrogen in South Korea to identify *Bacillus sp* isolates. Then the nucleotide sequence was compared with the data available on the NCBI website (http: // blast. ncbi.nlm.nih.gov/ Blast.cgi). plot phylogenetic tree of the isolates after matching them with strains in the gene bank using the MEGA program .

2.4 Preparation of mineral solutions

The storage solution was prepared by dissolving minute quantities of cadmium oxide in sterile, deionized distilled water. The working concentration of Cadmium was prepared from the appropriate serial dilution of the storage solution according to (**Etorki et al., 2013).**

2.5 Bioremoval Protocol

Bottles were prepared containing 100 ml of sterile nutrient broth and and Cadmium oxide with concentration of (0.5,0.2,0.1) mg/ml were added, individually and adjusted the pH to 7,distributed in tubes of 10 ml and added 0.5ml of bacterial growth to each tube so that (it contains 10^3 cells) and the tubes were incubated at 37 ° C for a period of 24,48,72 hours) **(Hietala and Roane, 2009**) After the incubation period was over, the centrifugation was done at 6000 rpm for 15 minutes, then filtered, and the concentrations were measured by Flame atomic absorption spectrophotometer **(Philip *et al*., 2000).**

3. Results and discussion

3-1- Isolation and diagnosis of *B. cereus*

Based on isolation steps and bacterial diagnosis, *B. cereus* bacteria were obtained and according to the apparent diagnosis, colonies of bacteria appeared after their growth on nutrient agar medium, white to creamy in shape with irregular edges from corrugated to filamentous as shown in Fig. (1**)**
(MacFaddin, 2000)

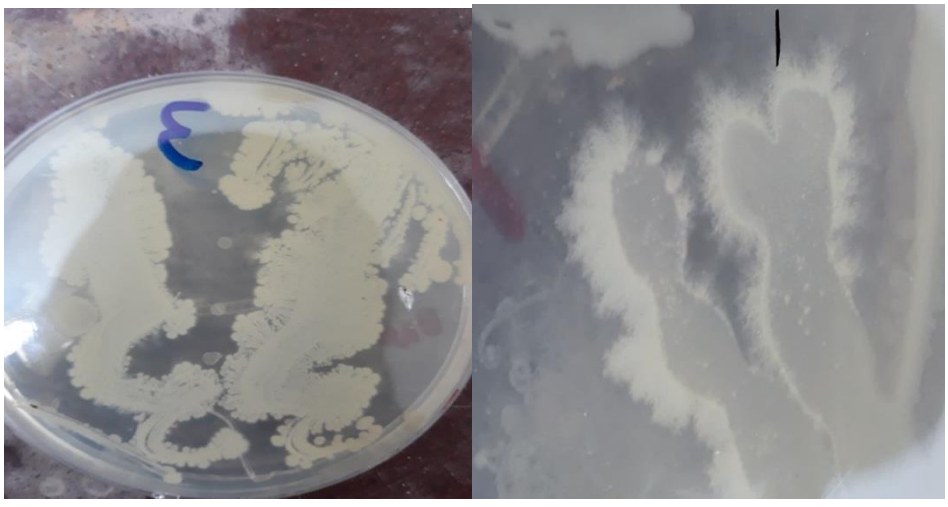

Figure (1): Phenotype of *B. cereus*

3.2 Molecular diagnostics

The results of agarose gel electrophoresis of DNA samples of DNA samples extracted from *Bacillus sp* isolates, and by using the primers of the 16 S rRNA gene, showed the DNA bundles were up to 609 base pairs as in Figure (2)

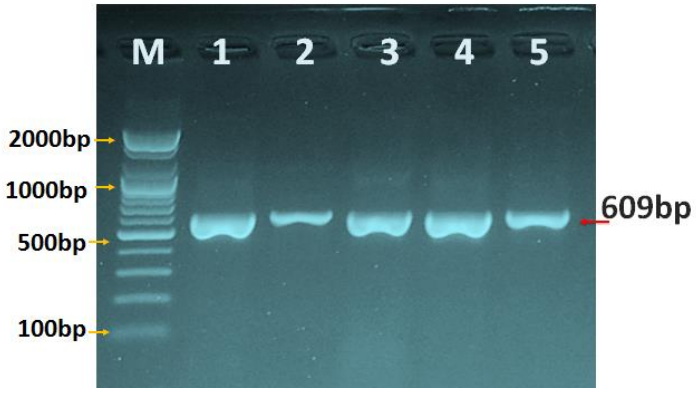

Figure (2) : Electrophoresis of Agarose gel showing PCR product of the 16SrRNA gene in *Bacillus cereus* Were M represents Marker, (1-5) represents sample numbers

3.2.1The genetic tree

After reading the RRNA 16S gene sequence and comparing it to the same gene recorded on the NCBI .site, the genetic tree was plotted using Mega 6 software

Figure(3): Multiple-sequence alignment analysis of the 16S rRNA gene partial sequence between native *Bacillus sp* soil isolates and *Bacillus* species in NCBI BLAST using MEGA software. Multiple alignment analysis showed similarity (*) and genetic difference (substitution mutation) in the 16S rRNA gene nucleotide sequence between isolates.

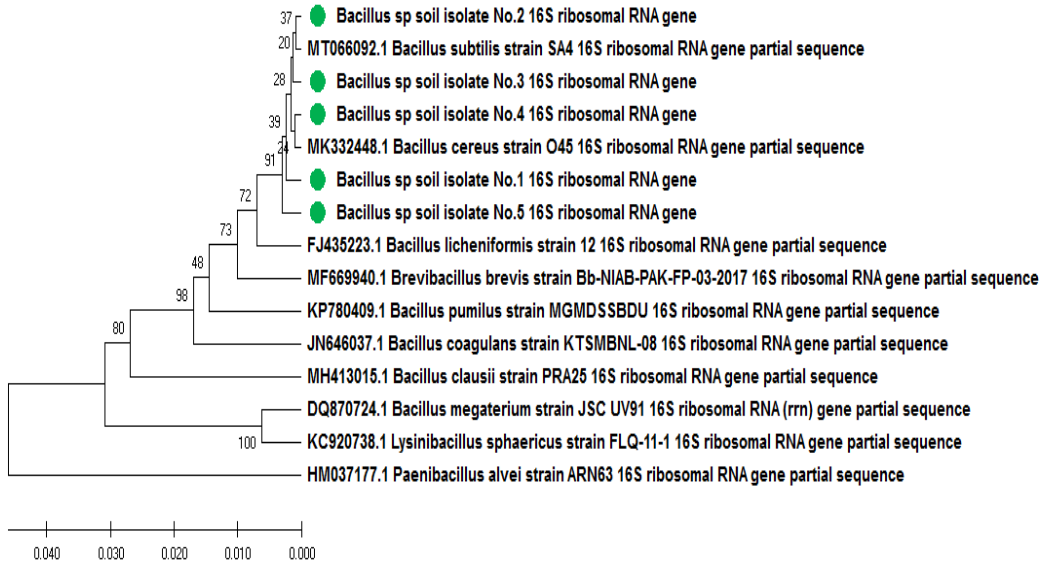

Figure (4) : Phylogenetic tree analysis based on partial sequencing of the 16S rRNA gene from local *Bacillus* Soil isolates used for genomic species identification analysis

3.3 Bioremoval experience

Table (1,2 shows the results of *B .cereus* that had a greater ability rate (86%) bacteria had a greater ability to remove cadmium that was at a concentration of 0.1 mg / ml and the lowest rate of removal (34%) was shown with concentration of (0.5 mg/ml)There is an inverse relationship between the removal percentage and the concentration of the element, where, the lower element concentration, corresponded the greater rate of removal. The reason for this is that at low concentrations, the heavy element present in the solution will find an opportunity to interfere and bond with sites **(Pandiyan and Mahendradas, 2011).** In the case of high concentrations, the active site is saturated with particles of the element, since this site is not able to bind to other metal ions **(Ray *et al* ., 2005; Tunali *et al*., 2006; Pandiyan and Mahendradas, 2001).**

The biological removal process does not depend on the type of bacteria but on other factors, including the number of active sites, the ease of access to these sites, the chemical status of the sites, and the proximity of the active sites to the minerals **(Viera and Volesky, 2000; Volesky, 2004).**

These results are in agreement with **(Pun *et al*., 2013; Ahalya *et al*., 2005; Nanganuru and Korrapari, 2012**). Incubation of the bacteria for 24 hours was better than 48 and 72 hours in removing the cadmium element due to the convergence of free metal ions to the binding sites on the wall of the bacteria, the process of removing the elements is highest in the beginning, but when these

sites become saturated, the removal process slows down and the removal does not increase Whenever the incubation period increases **(Mahmoud, 2006)** and the results are in agreement with **(Elham and Muhammad, 2017).**

Table (1). Results of bacterial activity of *Bacillus cereus* that used to remove cadmium in concentrations(0.1 , 0.2 , 0.5mg) within (72,48.24 hours)

concentration and time / species	concentration mg/ml	24 hour	48 hour	72 hour	control	LSD	P value
Bacillus cereus	0.1	0.132	0.275	0.345	0.986	0.057	0.002
	0.2	0.256	0.399	0.468	0.986	0.057	0.002
	0.5	0.380	0.647	0.723	0.986	0.057	0.002

Table (2): Percentages of cadmium removal by *B. cereus* bacteria

B .cereus				
Heavy Metals	time / concentration	hour 24	hour 48	hour 72
cadmium	0.1	86	72	65
	0.2	86	72	67
	0.5	61	39	34

Conclusions

1- *B. cereus* bacteria was isolated from agricultural soil

2- The optimum conditions for removing cadmium by *B. cereus* at pH = 7, a temperature of 35 ° C and an incubation period of 24 hours

3- The percentage of cadmium removal ranged from 34% to 86%

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