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BIO REMOVAL OF ARSENIC METALS FROM WATER SOLUTION BY *BACILLUS* SUBTILIS SP.

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ABSTRACT

The main routes of exposure to arsenic are drinking water, eating food, absorbing the skin and breathing the air. This exposure causes a range of human health problems. Bioremediation is an effective and environmentally friendly method. *B. subtilis* was isolated from different agricultural soils that were identified based on molecular diagnosis. The biological treatment process was carried out using *B. subtilis* bacteria and arsenic at concentration of (0.5, 0.2, 0.1 mg/ml) under pH = 7 conditions at 35 °C and for a period (24, 48, 72 hours), and an atomic absorption spectroscopy was used to analyze the concentration of arsenic after the completion of the treatment process. The results showed that the highest removal of arsenic was 86% at 0.1 mg/ml and the lowest removal by 56% at 0.5 mg/ml, and the best elimination period was at 24 hours.

KEY WORDS : *B. subtilis,* Arsenic, Bioremediation, 16S rRNA

INTRODUCTION

Among the most important pollutants are heavy elements in the environment, so their presence poses a great and important danger because their toxicity includes many life forms because they form non-degradable chemical complexes (Paknikar *et al*., 2003). Arsenic is one of the most toxic elements found in the environment as Arsine (III), Arsenic (0), Arsenite (III), Arsenate (VI) (Matschullat, 2000). Arsenic in the environment is not destroyed, but can be converted into other forms by reaction. With oxygen or other molecules in soil, water and air (Ghosh *et al.,* 2004). Due to the fact that heavy elements persist in the environment and are not degradable, it has become important to find an environmentally friendly option to clean the environment contaminated with minerals and thus preserve the health of the degraded environment (Sekhar *et al.,* 2004). Bioremediation is the process of using microorganisms or their enzymes to restore the environment changed by pollutants to its original state (Opeolu *et al.,* 2010). Among the various biological treatment options is the use of *Bacillus* sp. Its Gram-positive wall is characterized by the presence of a peptidoclycan layer. The secondary polymers are the most important of which are Teichoic acid and Teichuronicacid (Brooks, 2004). Heavy elements interact with active groups in the cell wall and membrane, as these groups are binding sites with elemental ions (Schiewer, 1996).

MATERIALS AND METHODS

Isolation of *Bacillus subtilis* from agricultural soil 2-1-

1g of moist soil was weighed and added to 10 ml of distilled water Samples were placed in a water bath at 60 °C for 60 minutes, after which dilutions of up to 10^{-3} were made for each dilution. A volume of 0.1 ml of each diluent was transferred aseptically to plates of nutrients and incubated at 37 °C for 24 hours (Aslim *et al.,* 2002).

Based identification 16S rRNA2-2-

16S rRNA gene was detected through DNA extraction from bacterial isolates, prefixes F (GTGAGGTAACGGCTCACCAA) were used R (CTTCAGCACTAAAGGGCGGA) manufactured by (Bioneer, South Korea). After the

completion of the PCR process, the results were sent to Macrogen in South Korea to identify *Bacillus* sp isolates. Then the nucleotide sequence was compared with the data available at the NCBI website (http: // blast. ncbi.nlm.nih.gov/ Blast.cgi). Then, a phylogenetic tree was drawn for the isolates after matching them with strains in the genebank and using Mega software.

Preparation of mineral solutions2-3-

An aqueous solution of AS_2O_3 arsenic oxide was prepared at a concentration of 100 mg/ml. This solution was used for the number of experimental solutions required (Etorki *et al.*, 2013).

Bioremediation Protocol 2-4-

flasks were prepared containing 100 ml of sterile Nutrient broth and concentrations were added (0.5, 0.2, 0.1 mg) of arsenic oxide individually and the pH was adjusted to 7, then distributed in tubes of 10 ml and adding 0.5ml of bacterial growth to each tube so that (it contains 103 cells) and the tubes were incubated at 37 °C for a period of (72, 48, 24 Hour) (Hietala and Roane, 2009) . After the incubation period was over, they were centrifuged at 6000 rpm for 15 minutes, then filtered, and the concentrations measured by atomic absorption spectrophotometer (Philip *et al.*, 2000).

Results and discussion3-

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

Based on isolation steps and bacterial diagnosis, B. subtilis 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).

Molecular diagnostics3-2-

The results of the electrophoresis showed on the Agarose gel of DNA samples extracted from Bacillus sp isolates, and by using the primer of the 16 S rRNA gene, the DNA bundles were up to 609 base pairs as in Fig. 2.

Experiment bioremediation 3-3-

Table 1, 2 the results showed that B. subtilis bacteria have the ability to remove arsenic at a concentration of 0.1 mg/ml at 86% and the lowest removal concentration is 0.5 mg/ml at 56%. The wall of B. subtilis contains peptidoclycans, Teichoic acid and Teichuronic acid. As the peptidoclycans contain quantities of the amino acid meso - DiaminoPimelic Acid, the carboxyl group of the amino acid not bind to the inser of the peptide (Slepecky and Hemphill, 2006). Teichoic acid is a negatively charged polymer, representing about 10 - 60% of the weight of the cell wall. It contains a high percentage of phosphates, has a metal-binding function and plays a major role in metal ion balance (Schaffer and Messner, 2005). Teichuronic acid containing hydroxyl groups that provide a negative charge to the cell wall (Beveridge *et al.*, 1982). These acids are important binding sites with metal ions (Pagnanelliet *et al.*, 2003). The negative charge on the cell surface allows the bacteria to bind to the positive ions of many heavy elements (Malekzadeh *et al.*, 2001), incubating the bacteria for 24 hours was better than 48 hours and 72 hours in removing the element arsenic, meaning that the removal was stable at a certain limit because there were no other binding sites on the surface of the bacteria (Tunila *et al.*, 2006; Ray *et al.*, 2005) .

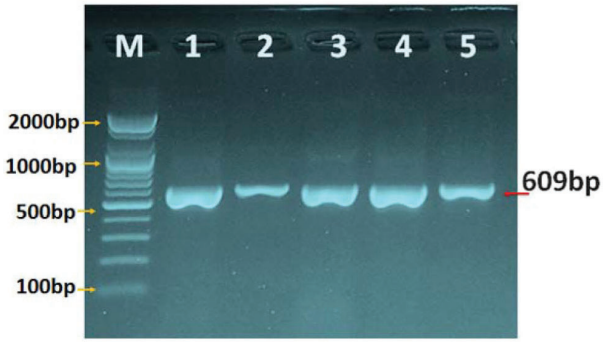

Fig. 2. Electrophoresis of Agarose gel showing PCR product analysis of 16SrRNA gene in *Bacillus* sp, (Number 2 represents bacteria *B. subtilis*)

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Table 1. Results of bacterial activity. *Bacillus subtilis* was used to remove Arsenic in concentrations(0.1 , 0.2 , 0.5mg) within (24,48.72 hours)

P value	LSD	Control	72 hour	48 hour	24 hour	Concentration µn g/ml	Concentration and Time Species
0.009	0.017	0.293	0.070	0.051	0.039	0.1	*Bacillus subtilis*
0.009	0.017	0.293	0.119	0.091	0.042	0.2	
	0.009	0.017	0.293	0.126	0.099	0.085	0.5

Table 2. Percentages of Arsenic removal by *B. subtilis* bacteria

	B. subtilis			
72 hour	48 hour	24 hour	Time concentration	Heavy elements
Arsenic	0.1	86	82	76
	0.2	85	68	59
	0.5	70	66	56

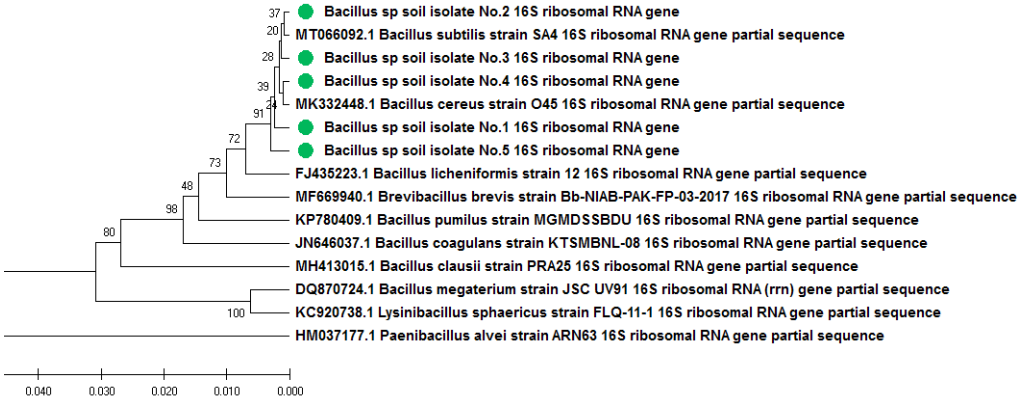

Fig. 3. Phylogenetic tree analysis based on the 16S rRNA gene partial sequence of local *Bacillus* sp. soil isolates that used for genetic species identification analysis. The phylogenetic tree was constructed using UPGMA method (MEGA X version), and the local *Bacillus* sp. soil isolates (No.2) were showed closed genetic related *Bacillus subtilis* strain SA4 (MT066092.1) at total genetic change (0.04-0.01%)

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