Isolation and identification of some chromium resistant bacteria from some contaminated sites

Athmar Ahmad Abdul Hussain1 Aamal Ghazi Mahdi Al-Saadi2

Department of Ecology – College of Science, University of Al-Qadisiyah, Iraq
Corresponding author e.mail: amal.alssdi@qu.edu.iq

Abstract

Heavy metals have fundamentally effect on the biological system and they are straightforwardly harmful to living creatures, particularly human. Expulsion of these pollutants requires advanced and exorbitant technologies. Along these lines, bioremediation with hefty metal-resistant microorganisms could fill in as a less expensive solution. The aims of this study were to select some chromium resistant bacteria in contaminated sites and to determine the inhibitory concentration values for each species. Selection of the chromium resistant isolates was done using nutrient agar plates containing 100mg/l of (K2Cr2O7), and the values of the inhibitory concentrations were determined by agar diffusion method. Strains that resisted the highest concentrations were diagnosed based on the morphological and molecular methods. Molecular diagnosis was performed based on the analysis of the sequence of nitrogenous bases of 16sr RNA gene as well as the generic tree relationship. Cr(VI) resistant isolates were chosen from a sum of 40 separates and analyzed as follows: Brevibacillus agri, staphylococcus sp., Bacillus sp., Pseudomonas sp., Exigobacterium sp., and Atlantibacter sp. The MIC values were 2500mg/l for Brevibacillus agri and staphylococcus sp. and 1500mg/l for the remaining species. In this paper, various bacteria strains, resistant to high chromium concentration, were isolated and identified, and these native microorganisms can be utilized for bioremediation of chromium.

Keywords: Chromium-Resistant Bacteria, Heavy metal, pollution, Hexavalent chromium.

1-Introduction

A contamination is defined as a substance that is normally delivered in nature by human exercises and has destructive effects on the environment [1]. Poisons can be separated into two significant gatherings [2], the first group includes pollutants that physically impact the environment and the second group contains those that are straightforwardly harmful to living beings, especially people [3],[4]. The development and move of toxins in nature is known as contamination, and it makes various dangers human wellbeing and other living organisms[5]. Numerous efforts are made to remove pollutants so that they can be discarded prior to entering environment, or subsequent to entering the environment, or changing over them into safe and low-risk materials [6]. Heavy metals are amongst these substances that can cause serious health problems. These materials are frequently found in diverse oxidation states in environment [7]. Because of the impact, both short-and long haul advantages of these minerals are examined, with a background marked by acceptable foundations for these
minerals in drinking water and in mechanical and metropolitan wastewater [6]. Among the entirety of the heavy metals chromium is considered incredibly harmful. It was first found in 1998 by a French scientific expert called Vauquelin[8]. The destiny of chromium in nature is subject to its oxidation state. The hexavalent form of chromium (VI) is easily bioavailable because of its high dissolvability, while the trivalent form of chromium (III) is much less poisonous, less soluble under neutral pH, and unable to cross cell membranes[9],[10]. Hexavalent chromium (VI) has been determined as a huge poison by the US EPA because of its cancer-causing nature, teratogenicity and mutagenicity for mammals including people. It is perhaps the most generally utilized materials in industry, bringing about extraordinary amounts being released into the environment [11]. Chromium is existing in modern squanders basically in the hexavalent form as chromate and dichromate. The principle sources of chromium incorporate the metal completing industry, petrol refining, cowhide tanning, iron and steel ventures, inorganic synthetic substances creation, material assembling and mash delivering. Furthermore, chromium compounds are added to cooling water to forestall erosion and are contained in certain additives and fire-retardant synthetics utilized in wood preservatives[12],[13]. The chromium releases from these modern cycles was assessed at roughly 10000 lbs each day by the US EPA. Rather than most metals, chromium is profoundly water dissolvable and versatile in the environment. Thus treatment to lessen or eliminate this contamination before removal into the environment is necessary[8]. Generally hexavalent chromium is treated by various physical and chemical methods like reduction, precipitation, adsorption, particle trade, adsorption, invert assimilation, and electrodiallysis that are economically costly and has disadvantages like incomplete metal expulsion, high reagent utilization, generation toxic sludge, and generation toxic sludge [14],[15]. Furthermore, the vast majority of these techniques additionally have a few constraints they are like practicality at high or moderate concentration of metals yet not low concentration. Inerable from the aforementioned challenges, bioremediation strategy for substantial metal detoxification has more extensive implications[14]. Thus, incredible interest in metal microorganism communications has known in the previous few decades, as specialists and industrialist solicitation to eliminate, recuperate or balance out hefty metals in soil and effluents[5],[15]. Albeit significant degrees of chromium (VI) are poisonous to most organisms, the bioremediation by microorganisms is viewed as the most reasonable strategy since some bacterial populace can show resistance to from as much as 1000mg/l of Cr (VI)[2] [16]. Chromium remediation through microorganisms is acknowledged as the best and financially affordable technology at present to spring clean Cr contamination[17]. Microbial cells are known to have the option to foster protections from for all intents and purposes all toxic heavy metals. Bacterial resistance to chromate may be plasmid-borne or due to chromosomal mutations[18]. The aims to this study were to seclude, analyze and recognize of chromium resistant bacteria in contaminated sites and determine the inhibitory concentration values for each species.

2-Materials and Methods

2.1- Sample collection:

Samples were congregated from the diverse contaminated sites, including: Diwaniya material and calfskin processing plant, oil purifier, Al-Bahar vegetable oil plant, Agrarian wastewater, Elastic lab – Diwaniya, Tire reusing , The state Organization for Material and Cowhide
Industry, Baghdad, Al-Maamoun Industrial facility Plastic industry, Babylon Battery Plant, Gem colors research center, The Shanafia Processing plant, AL-Ghadeer cleansers production line, Al-Wazzan cleansers manufacturing plant, Synthetic labs, Clinical labs and waste water for carwash. The samples were collected in clean plastic cylinders and immediately to the research center with ice box.

2.2-Bacterial selection:

The samples were inoculated on supplement nutrient agar then incubated at 37°C for 24 hours. At that point inoculum of each culture was plated on supplement agar and grown at 37°Covernight. The chromium resistant test of bacterial isolates was performed utilizing 1ml/g of an sample, which then was blended with 9ml of serially physiological saline. Sequentially dilutions were made, and 0.1ml from each dilution was spread on supplement agar plates containing 100mg/l of (K2Cr2O7). The development of the bacterial colonies was seen after 24h of incubation at 37°C.

2.3-Identification of strains:

Diverse bacterial colonies acquired after incubation were filtered and stored at 4°C. Bacterial strains were examined under the microscope after Gram staining, and the colonies morphological were observed. Molecular identification of the selected bacteria was recognized by PCR amplification of 16S rRNA utilizing universal forward primer (5-AGAGTTTGATCCTGGCTCAG-3) and reverse primer (5-GGTTACCTTGTTACGACTT-3) [19]. The amplified gene was then sequenced and the subsequent 16S rRNA gene sequences were contrasted and arrangements saved in GenBank. A similarity look for the nucleotide succession of 16S rRNA of each seclude was completed using the NCBI nucleotide BLASTtool. The 16S rRNA sequence was submitted to NCBI GenBank for registration.

2.4-Determination of inhibitory concentration of Cr (VI):

The minimal inhibitory concentration (MIC) of Cr (VI) was determined by growing the secludes on nutrient agar medium enhancement with various concentrations 200, 400, 500 ,700, 800,900,1000,1500,2000 ,2500 and 3000mg/l of chromium with control (without chromium). The plates were grown at 37 °C for 24 hours. The zone of inhibition was recorded for every concentration. The zone of inhibitions under 1 mm was regarded as the inhibitory concentration of the chromium (K2Cr2O7) MIC [20].

3-Results and discussions

3.1-Identification and characterization of the highest Cr resistant isolates:

Forty bacterial strains were confined from the polluted locales that examined through this study. 20 of these were read for their capacity to resist high chromium concentration and distinctive phenotypic qualities are listed in (Table1) as per Bergey's manual of determinative bacteriology and molecular identification. Just 8 were distinguished which were impervious to high concentrations. molecular ID was made dependent on the investigation of the sequence of nitrogenous bases of 16SrRNA gene and the phylogenetic tree (Figur1; 2). Results shows extremely undeniable degree of resistant against Potassium dichromate in supplement nutrient agar likewise significant degree of safe was additionally announced from polluted destinations [30]. Various past reports proposed that 16S rRNA quality succession
investigation is better when looked at than ordinary phenotypic strategies in recognizing microscopic organisms (35; 36).

The PCR enhanced result of 1500 bp of DNA portion shows the 16S rRNA quality intensification in the picture created by gel documentation framework Fig. 1. Comparative strategies used to distinguish chromium tolerant microorganisms from soil tests in electroplating industry (37). These organisms were also distinguished by 16S rRNA quality succession and a similar strategy used to recognize the Microbes species. In this examination, the microbes were identified as *Brevibacillus agri*, *staphylococcus* sp., *Pseudomonas* sp., *Exigobacterium* sp., and *Atlantibacter* sp. And three strains of *Bacillus* sp. The confined microscopic organisms showed 100% likeness with reference grouping recovered from NCBI (Fig 2).

![1500 bp](image)

Fig 1: Agarose gel electrophoresis image that showed the PCR product analysis of 16S ribosomal RNA gene isolates. Lane 1:Marker ladder (1800-100bp), lanes (2-10) positive 16SrRNA gene isolate at 1500bp PCR product size.

3.2-Morphological properties:

In light of the phenotypic qualities of separates under magnifying instrument after Gram staining and as indicated by the contrasting and Bergey's manual of determinative bacteriology (Table 1).

Table 1: Morphological properties of the selected chromium resistant isolates

| Isolates | Bacterial name       | Morphological properties                                      |
|----------|----------------------|----------------------------------------------------------------|
| 1        | *Bacillus* sp.       | Gram positive, rod shape, and white opaque color.              |
| 2        | *Bacillus* sp.       | Gram positive, rods shape and off-white color.                 |
| 3        | *Brevibacillus agri* | Gram negative, rod shape, and colorless.                       |
| 4        | *Staphylococcus* sp. | Gram positive, spherical in shape, and grayish white color     |
| 5        | *Exigobactrium* sp.  | Gram positive, rods, and with orange color.                    |
| 6        | *Bacillus* sp.       | Gram variable, rod, and cream opaque color.                    |
3.3-Determination of inhibitory concentration of Cr (VI):

MIC for secludes were resolved utilizing chromium concentrations. The outcomes showed that the *Brevibacillus agri* and *Staphylococcus* sp. showed the highest resistant to chromium. The insignificant inhibitory concentration of the separated bacterial strains are appeared in table 2 and figure 3.

| Sr. No | Bacterial Isolates       | Minimum inhibitory concentration (MIC) |
|--------|--------------------------|----------------------------------------|
| 1      | *Bacillus sp.*           | 1500 mg/l                               |
| 2      | *Bacillus sp.*           | 1500 mg/l                               |
| 3      | *Brevibacillus agri*     | 2500 mg/l                               |
| 4      | *Staphylococcus sp.*     | 2500 mg/l                               |
| 5      | *Exigobactrium sp.*      | 1500 mg/l                               |
| 6      | *Bacillus sp.*           | 1500 mg/l                               |
| 8      | *Atlantibacter sp.*      | 1500 mg/l                               |
| 10     | *Pseudomonas sp.*        | 1500 mg/l                               |
Figure 2: phylogenetic tree the 16S rRNA gene sequence of strains.
Chromium resistant bacteria were recognized as *Bacillus* spp and *Staphylococcus* spp as indicated by Bergey's manual Efficient Bacteriology. Both disengages showed high impervious to Cr (VI).

Potential microorganisms particularly bacterial species can eliminate substantial metals from arrangements by biosorption or bioaccumulation or both. An assortment of instruments exist for the expulsion of substantial metals from polluted sites by microbes, growths, ciliates, green growth, greeneries, macrophytes and higher plants [13,14].

Biosorption generally includes actual adsorption followed by synthetic servitude and doesn't need energy. Once, the metal particles are diffused on the cell surface, they tie to destinations, which show synthetic fondness for the metal. It is a latent collection measure, which may incorporate adsorption, particle trade, complexation, chelation, and microprecipitation.

During the current examination *Bacillus* spp and *Staphylococcus* spp both were discovered to be exceptionally tolerant to chromium, Cr(VI). They are portable in the environment in light of their high dissolvability in water. The two microorganisms did not just show the capacity to make due in sullied wastewater yet in addition exhibited a checked expanded in remediation of poisonous Cr (VI) in their essence. A few analysts have likewise revealed the immediate decrease of Cr (VI) in debased effluents of the metal completing industry[16]. One potential technique was microbially catalyzed decrease of Cr (III), which was first revealed.
with Pseudomonas spp.[17]. From that point forward, huge advancement has been made towards understanding the cycles controlling enzymatic decrease of Cr (VI) in Gram-negative microorganisms, particularly those having a place with the genera *Pseudomonas*, *Desalfovibrios* and *Shewanella* [18]. A few Gram-positive microorganisms are additionally known to diminish Cr (VI) including a few individuals from the sort Bacillus [19]. The dividers of Gram positive microbes are effective metal chelators and in *Bacillus subtilis*, the carboxyl gathering of the glutamic corrosive of peptidoglycan was the significant site of metal statement. Teichoic and teichuronic acids were significant restricting locales in *Bacillus licheniformis* [20].

*Staphylococcus* spp is likewise a Gram positive bacterium and it has comparative cell divider properties as of other Gram-positive microbes. The conceivable system of hexavalent chromium decrease by microscopic organisms, segregated from tannery wastewater has been assessed. *Bacillus* spp and *Staphylococcus* spp showed phenomenal capacity to lessen hexavalent chromium to non harmful trivalent chromium, for example 86% and 74%. Thus, both the secludes have been distinguished as expected microorganisms for its helpfulness in eliminating chromium from the tannery gushing. The innovation when updated will be an aid to leather treaters in handling the contamination issue of tannery wastewater. The interaction would not exclusively be a practical yet in addition eco-accommodating and maintainable.

**Conclusion:**

Cr(VI) is portable in the enviroments as a result of its high solvency in water. Openings to ecological Cr have prompted the advancement of Cr-safe microscopic organisms. Here, we have detailed the presence of Cr-safe microorganisms in the squanders. Fruitful detachment of eight chromium-safe bacterial strains was refined. The strains distinguished as *Bacillus* sp.s, *Pseudomonas* sp., *Staphylococcus* sp., *Exigobactrium* sp., *Bacillus* sp., *Brevibacillus agri*, *Atlantibacter* sp. and *Bacillus* sp. These microorganisms can be utilized as bioremediation to chromium contamination.

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