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BIOREMEDIATION OF CADMIUM CONTAMINATED SOIL USING BACTERIA

Abhishek Kumar Pandey[1]*, Abhilasha Shrivastava[2] and Rahasya Mani Mishra[1]

[1]Center for Biotechnology Studies, Awadhesh Pratap Singh University Rewa - 486003, MP, India.
[2]Department Biotechnology, Government Model Science College, Rewa - 486003, MP, India.

***Corresponding Author: Abhishek Kumar Pandey**

Center for Biotechnology Studies, Awadhesh Pratap Singh University Rewa - 486003, MP, India.

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ABSTRACT

Bioremediation is the use of living organisms (primarily microorganisms) for removal of a pollutant from the biosphere. It relies on biological processes to minimize an unwanted environment impact of the pollutants. The microorganisms in particular have the abilities to degrade, detoxify and even accumulate the harmful organic as well as inorganic compounds. Soil samples were collected from J P Cement Factory, Rewa from different places at a depth of 6 -10 Inches from 4-6 different spots. These soil samples were mixed properly and enriched for cadmium resistant clones by incubating 10g of slag in 90 ml of sterile water amended with 10 ml Luria Bertani (LB) medium and 20 µg/ml each of cadmium chloride at 37°C for 2h. Supernatants were plated at 10^{-2} dilution by spread-plate method on LB agar medium. The plates were then incubated at 37°C. The colonies appeared after 3 days. Strains were preserved and phenotypic studies were carried out. The metal accumulation efficiency was measured by Atomic Absorption Spectroscopy (AAS). The soil samples collected are all alkaline in nature, the isolated bacteria are gram negative, short rod, aerobic bacteria and according to this research sample with bacterial inoculation in them shown reduction in the cadmium levels as compared to the raw soil samples.

KEYWORDS: Bioremediation, Heavy metals, Soil, Bacteria.

INTRODUCTION

Contamination of heavy metals in the environment is a major global concern, because of toxicity and threat to the human life and ecosystem. The levels of metals in all environments, including air, water and soil are increasing in some cases to toxic levels, with contributions from wide variety of industrial and domestic sources. Metal contaminated environments pose serious threat to health and ecosystems. Metals like arsenic, cadmium, lead, mercury etc cause conditions including hypophosphatemia, heart disease and liver damage, cancer and neurological and cardiovascular diseases, central nervous system damage and sensory disturbances. Bay incident of Japan is an example of heavy metal poisoning which occurred due to consumption of fishes and shellfishes contaminated with methylmercury in their body.[1]

Because of toxicity and the ubiquity of the metals in environment, microbes have developed unique and sometimes bizarre ways of dealing with unwanted metals. Some microorganisms have mechanisms to sequester and immobilize metals, whereas others actually enhance metal solubility in the environment.[2] Sometimes they oxidize or reduce them to a non-toxic or relatively less toxic forms. Bioremediation is a sustainable strategy that utilizes the metabolic potential of microorganisms and plants to clean-up contaminated environments. It achieves contaminant decomposition or immobilization by exploiting the existing metabolic potential of microorganisms with novel catabolic functions derived from selection or by introduction of genes encoding such functions. Bioremediation is a cost effective eco-friendly means of healing nature with nature. This technology may be applied in the removal of xenobiotic compounds from agrochemical and petrochemical industries, oil spills, heavy metals in sewage, sludge and marine sediments etc.[3]

MATERIALS AND METHODS

Collection of soil samples - Soil samples were collected from J P Cement Factory, Rewa from different places at a depth of 6 -10 Inches from 4-6 different spots.

Physicochemical characteristics - Different physico-chemical characteristics of the leachate such as pH, Dissolved Oxygen (DO), alkalinity and total water content were determined for both freshly produced slag and old slag deposited considerably since long period of time. Methodologies followed were according to American Public Health Association (APHA).[4]

Strains isolation –The samples were mixed properly and enriched for cadmium resistant clones by incubating 10g of slag in 90 ml of sterile water amended with 10ml Luria Bertani (LB) medium and 20 µg/ml of cadmium

chloride at 37°C for 2h (Higham and Sadler *et. al*.1984). Supernatants were plated at 10^{-2} dilution by spread-plate method on LB agar medium containing 20 µg/ml of Cadmium Chloride [$CdCl_2$]. The plates were then incubated at 37°C. The colonies which appeared after 3 days of incubation were further screened at higher concentrations (20-800 µg/ml) of heavy metal. Finally, the strains were selected as cadmium resistant isolates for further studies.

Maintenance and preservation of cultures

Strains were preserved in the refrigerator in stab cultures made of LB medium and NA medium both for short time preservation. The media were all the time supplemented with 20 µg/ml of heavy metal and for long time preservation glycerol stocks were made for storage in –70°C for longer period of time. For this overnight grown liquid culture were taken in cryo-vials and added with 15% glycerol after this the vials were transferred to –70°C deep freezer.

Phenotypic studies and Determination of Maximum Tolerance Limit (MTL)

Phenotypic studies such as colony morphology, Gram staining, endospore staining, motility, biochemical tests, acid production tests etc. were carried out as per standard methods of Benson, 1990 in our laboratory.[5] MTL was determined by growing cells both in LB broth and LB agar media which had been amended with increasing concentrations of respective heavy metal. Heavy metal (Cd) stock were prepared in sterile distilled water and was added at the time of inoculation of specific bacterial isolates in inoculating chamber.

Heavy metal accumulation efficiency measurement

The heavy metal accumulation efficiency was measured by Atomic Absorption Spectroscopy (AAS). For this 500 mg cell pellets obtained at different time intervals were suspended in 20 ml water added with 5% conc. HNO_3 and 0.5% conc. HCl. The cell suspensions were then digested in Anton Paar MDS according to User 001H of Perkin Elmer Application Note (Instruction Manual, Perkin Elmer AAnalyst 700). Following digestion, the cell extracts were analysed in AAS.

RESULTS AND DISCUSSION

The physico-chemical characteristics indicate that the place is unsuitable for the growth of plant mainly because of high pH. The older slag is more alkaline than the slag deposited recently. The dissolved oxygen of the older slag is also considerably higher than the fresh slag.

The cadmium resistant strain is a gram negative, short rod, aerobic bacteria. To study the maximum tolerance limit (MTL), cells were grown in LB broth with increasing concentrations of heavy metals and growth was observed, the maximum tolerance limit of cadmium resistant bacteria was 130 µg/ml. The concentrations of heavy metal were determined using Atomic Absorption Spectroscopy (AAS) as described in materials and methods. The concentrations were determined at 12hrs, 18 hrs, 24 hrs and 48 hrs of growth. it can be seen that the accumulation of heavy metal (Cadmium) increases gradually in log phase and they show maximum level of accumulation at late log phase to stationary phase (i.e. at 18-20 hrs), however, the concentrations of heavy metals decrease when the cells enter stationary to death phase. The maximum accumulation efficiencies of cadmium resistant strain as determined by atomic absorption spectroscopy were 0.197 mg/g (Figure 1).

The principal purpose of this study was to isolate and characterize some heavy metal resistant bacteria from slag with respect to their heavy metal accumulation efficiencies. As has been discussed earlier that after a few rounds of screening at consecutively increasing concentrations of cadmium, bacterial isolates were screened and selected for further studies.

The alkaliphilic nature and salt tolerant abilities of the strains are significant with respect to their application in metal contaminated, salinated alkaline soil for bioremediation. Moreover, the slag, the natural habitat from which these bacteria were isolated also had a pH of 9.8.[6] The increased metal tolerance in relation to pH has been reported in *Aspergillus* sp.[7]

The cadmium accumulation efficiency of the strain as determined by atomic absorption spectroscopy was found to be optimum at 18 hours after inoculation when the population reached almost the maximum density. The accumulation efficiency then decreased considerably indicating it to be an active uptake system requiring energy for transport across the membrane.[8]

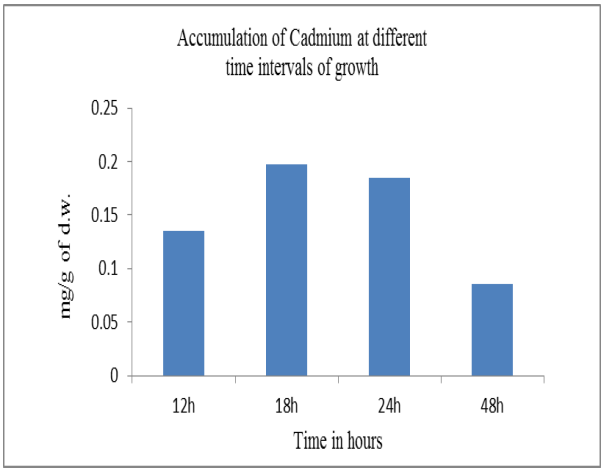

Figure 1: Heavy metal accumulation efficiencies at different time intervals.

It has been shown that microbes have the capability to reduce heavy metals, but whether they reduce it for detoxification or for growth is a matter of concern. In a sample of agricultural soil, arsenate reduction was not coupled to growth.[9] rather it could be linked to detoxification. However, in the JMM-4 strain of *Bacillus* sp., a close relative of *Bacillus arsenicoselenatis* isolated

from arsenic contaminated mud in Australia had been capable of oxidizing lactate to acetate while reducing arsenate to arsenite.[10] Regarding lead resistance there are reports that level of resistance for lead is also directly related with the biomass which is in accordance with our study.[11]

CONCLUSION

Conclusively, the bacterial isolates isolated from the slag disposal site of JP Cement factory Rewa, MP, India have been characterized in the present study with respect to their heavy metal acquisition and resistance and according to this research sample with bacterial inoculation in them shown reduction in the cadmium levels as compared to the raw soil samples. Thus it has been concluded that bioremediation is a potential method for solving the problem of heavy metal pollution.

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