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RESEARCH ARTICLE
Nagendram Kattepogu et al, The *Experiment, 2015., Vol. 33(2), 2090-2095*

ISOLATION AND SCREENING CHROMIUM REDUCING BACTERIA FROM INDUSTRIAL EFFLUENTS AND POLLUTED SOIL

Abstract:
In the past few decades, the environmental pollution of toxic heavy metals is the major issue throughout the world since industrial evolution. Chromium is one of the heavy metal whose concentration in the environment is increasing due to different industrial processes. Bioremediation is the most promising and cost effective technology widely used now a days to clean up both soils and wastewaters containing organic or inorganic contaminants. A variety of microorganisms have been known for their ability to degrade these heavy metals. The current study aimed to isolate Chromium reducing microorganism from industrial effluents and polluted soil. Among all isolated species three bacterial strains have exhibiting high level of resistant to Hexavalent Chromium salts. These three bacterial strains such as SS125, SS31 were identified by various biochemical tests. Then these three strains were further studied for chromium reducing activity and effectiveness of the bioremediation was evaluated. Based on the bioremediation efficiency, the strain (V3) Pseudomonas sp. was selected for the detoxification experiment which exhibits maximum Hexavalent Chromium reduction. Hence we conclude that isolate SS125 has high potential Chromium reducing activity among all isolates and further optimization studies are required to enhance the reducing activity.

Key-words: Environmental Pollutant, Effluent, Hexavalent Chromium, and Chromium Reduction.

1.0 Introduction:

Environmental contamination is increasing day by day because of increase in population, industrialization and urbanization [1]. Mining and industrial activities, and automobile exhausts are leach into underground waters, moving along water pathways and eventually depositing in the aquifer, are washed away by run-off into surface waters thereby resulting in water and subsequently soil pollution [2, 3]. The biotoxic effects of heavy metals occur when the body consumed above the bio-recommended limits. Although individual metals exhibit specific signs of their toxicity [4]. Chromium is a naturally occurring element found in rocks, animals, plants, soil, and in volcanic dust and gases. Chromium is present in the environment in several different forms. The most common forms are Chromium (0), Chromium (III), and Chromium (VI). Because of its toxic nature, Cr creates numerous environmental problems in waste products, mine wastes, and post manufacturing slag piles. It may cause carcinogenic (causing cancer) and noncarcinogenic diseases. The clinical features of acute poisoning are vomiting, diarrhea, hemorrhage and blood loss into the gastrointestinal tract, causing cardiovascular shock [5, 6].

Bioremediation is the use of living organisms, primarily microorganisms, to degrade environmental contaminants into less toxic forms. It is the use of microorganism metabolism to remove pollutants [7-9]. Bioremediation can occur on its own (natural attenuation or intrinsic bioremediation) or can be spurred on via the addition of fertilizers to increase the bioavailability within the medium (biostimulation). Bioremediation can remove pollutants relatively low cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site. Microorganisms used to perform the function of bioremediation are known as bioremediators [10]. Few micro organisms in the environment have been identified as potential chromium removal capacity [11-16]. Because of the wide range of exposure of chromium in the environment the need of finding potential microbes having potential Chromium immobilization property, the present study is aimed to isolate the Chromium reducing bacteria.

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2.0 Materials and Methods:

2.1 Instrumentation

Teccomp UV-2301 double beam UV-Visible Spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Standard cuvettes of 10mm path length are used for analysis. Standard Chromium was weighed by using Denver electronic analytical balance (SI-234). Autoclave (), Laminar air flow (), Rotary shaker () and Centrifuge () were used.

2. 2 Chemicals and Reagents:

All the chemicals used for preparation of growth media for isolation and optimization of Bacteriological grade, Merck chemicals limited, Mumbai. Standard $K_2Cr_2O_7$, coloring reagent Diphenyl Carbazide and other chemicals used were of AR grade and were purchased from Loba chemicals, Mumbai.

2.3 Collection of samples:

Soil and water samples were collected from different Chromium pollutant location near Budampadu, Guntur, AP. Industrial Effluent Samples were collected from NSL textiles Chandol, Guntur district, AP. All samples were collected in sterile tubes and transferred to the laboratory.

2.4 Isolation of Cr (VI) resistant bacteria:

Isolation of Cr (VI) resistant bacteria were carried out using Nutrient agar medium supplemented with different concentrations of Cr (VI). Soil samples were suspended in sterile distilled water and serial dilution were prepared using same water. The collected water samples were directly used for isolation of bacteria. Aliquots (100 µl) of different dilutions were plated on the agar medium and incubated at different incubation temperatures for several days. The obtained colonies were sub-cultured several times in fresh agar media until single homogeneous colonies were obtained.

2.5 Evaluation of chromium resistance:

The isolated Cr resistant bacteria were further screened for maximum reducing activity. Nutrient agar medium containing different concentration range of 1µg/ml to 1000µg/ml of Cr concentration were used for screening the reduction activity. The organisms which are grown in the higher concentration were selected for further activity study.

2.6 Estimation of Chromium reducing activity by spectrophotometer:

Diphenyl Carbazide method was followed for the estimation of Cr reducing activity by Visible Spectrophotometer. To 1ml of Cr solution, 1ml of 1N HCl solution was added and mix the solution and then 1ml of Diphenyl Carbazide solution was added. The content was mixed well, wait for 5min to develop purple color. The optical density of the obtained color was measured at 540nm against reagent blank. The procedure was repeated for different aliquots of standard Cr solution and calibration curve was constructed using concentration against absorbance forum. The calibration curve was used for estimation of Cr in samples. LB broth with 1000µg/ml of chromium concentration was prepared and 50ml of broth is taken into 100ml of Erlenmeyer flasks. Isolates those are capable of grown in 1000µg/ml were selected and loopful of inoculum was inoculated in to Erlenmeyer flasks containing 50 of LB broth and kept for incubation. After incubation cells were collected after centrifugation at 10,000rpm for 10 minutes. Then supernatant was analyzed for residual chromium by 1, 5- Diphenyl Carbazide method by measuring absorbance at 540 nm using a spectrophotometer. Similarly in order to observe chromium reduction, sediment of bacterial cells were washed twice with Phosphate Buffer Solution (PBS) and resuspended in water and presence of Chromium was observed Diphenyl Carbazide DPC method.

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2.7 Identification and characterization of the best Cr resistant isolates

Three best Cr resistant isolates determined by quantitative studies were identified up to genus level by studying phenotypic characters and biochemical studies like gram staining, motility and biochemical characteristics like oxidase, catalase, IMVIC and selective medium. All these results were compared with Bergey's manual of determinative bacteriology to determine the genus.

3.0 Results and Discussions:

3.1 Isolation and screening of Cr-resistant bacteria:

Five Effluent, soil and water samples were screened for Cr resistant isolates. After incubation based on the morphological and growth characters 31 different bacterial isolates were separated and sub cultured in nutrient agar medium followed by Cr resistant screening. Cr resistant screening was conducted by gradually (100, 250, 500 1000µg/ml) increasing the Cr concentration on the medium streaked with isolates. Growth of bacteria on chromium containing plates indicates positive growth i.e. Chromium resistance activity. And no growth indicates the negative i.e. Chromium sensitive organisms. After screening among 17 isolates 10 were capable of grown in 1000µg/ml Chromium concentration medium. 7 (seven isolates were sensitive to Chromium and growth was not observed on plates with 1000µg/ml Chromium.

3.2 Determination of Cr (VI) reduction activity

Reduction of Chromium levels were observed in supernatant from broth inoculated with the five isolates. This indicates that the isolates are capable of immobilization or reduction of Chromium from the broth. Reduction of Chromium levels were not observed in five isolates. This indicates that these isolates were capable of grown in presence Chromium due its resistance activity. But they not capable of immobilization or reduction of Chromium. Presence of Chromium residue in the sediment (pellet) of positive tubes when react with the 1, 5- Diphenyl carbazide by formation of violet indicates the presence of Chromium. This indicates the conformation of immobilization of Chromium by bacterial isolates. Based on the amount of Chromium found in the broth supernatant percentage of Chromium reduction was estimated and it was found that the bacterial strains coded SS215, SS31 were capable of high reducing activity (58%, 63% respectively) than other three strains.

3.3 Biochemical characterization of isolated bacterial strains:

The isolated bacterial species were identified following the Bergey's Manual of Determinative Bacteriology. The identification criteria included the growth features (colony, shape and color), growth conditions (optimum temperature and suitable growth media), morphology of the cells (shape), physiological characteristics (motility, flagella and Gram reaction), production of enzymes (oxidase and catalase), and utilization of different carbon sources. Results of Biochemical tests are listed in table-1. It was found that the bacteria were circular gram negative.

4.0 Conclusion

The present study concluded that indigenous bacterial species from polluted samples and effluents have their naturally existing machinery to degrade pollutants (Chromium), which is cost effective as compared to conventional methods. Two different bacterial species were isolated are identified that they have high degrading ability for Cr (VI) and have significant potential to degrade the toxic Hexavalent Chromium. These findings are potentially useful because the species can possibly be harnessed to detoxify chromium contamination sites and further optimization studies are required to optimize characters of the bacteria that can reduce high Chromium concentration.

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Figure A: results of isolation

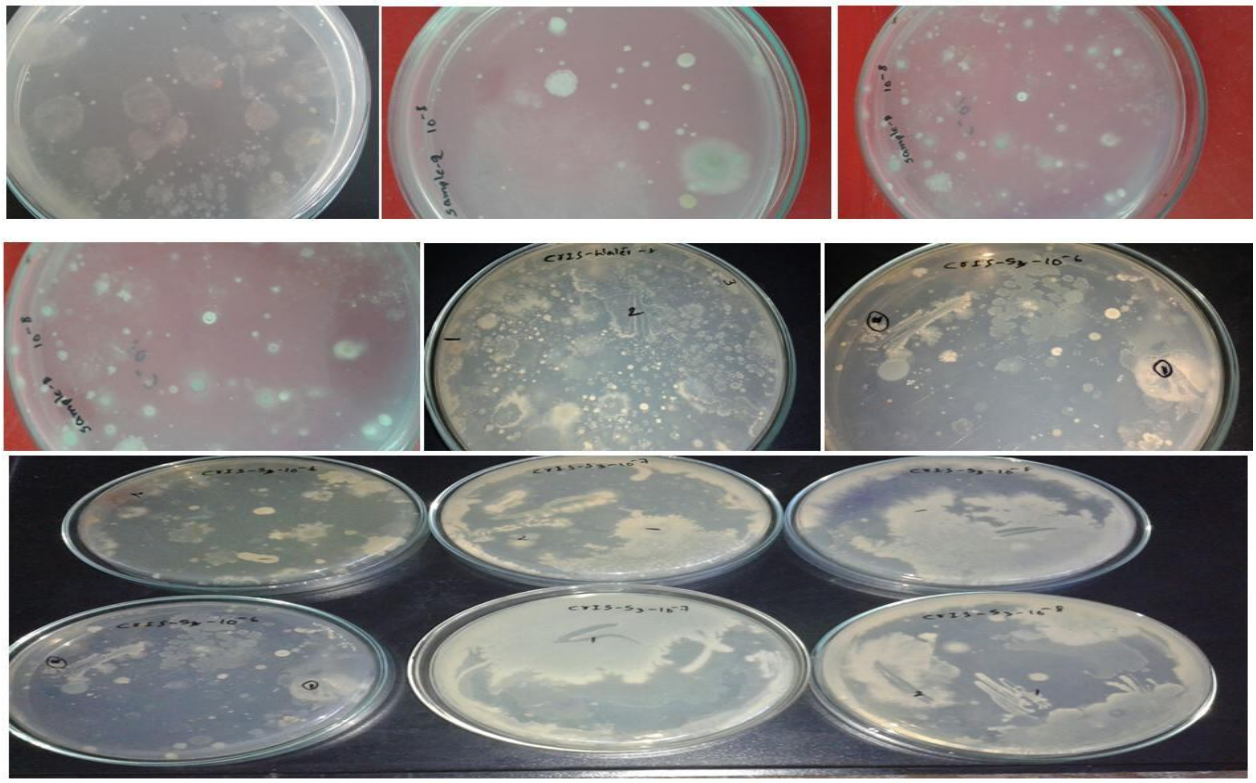

Figure B: biochemical studies results

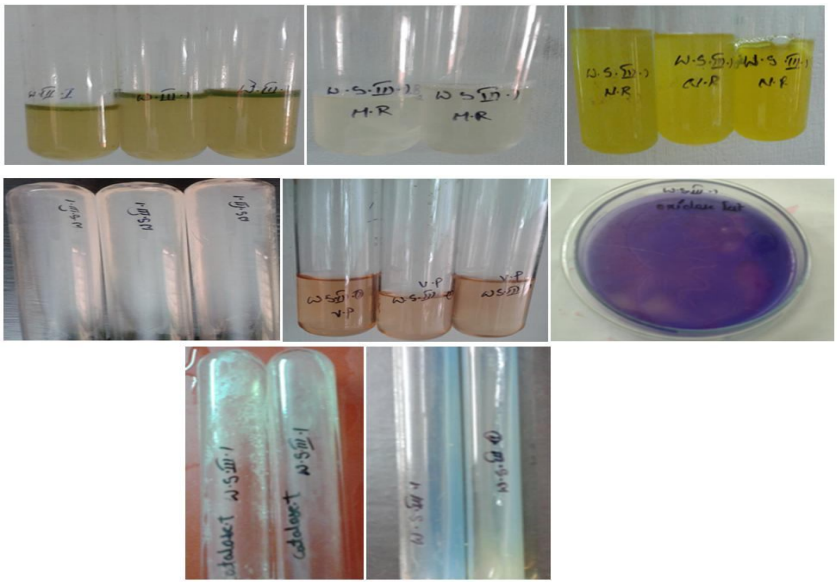

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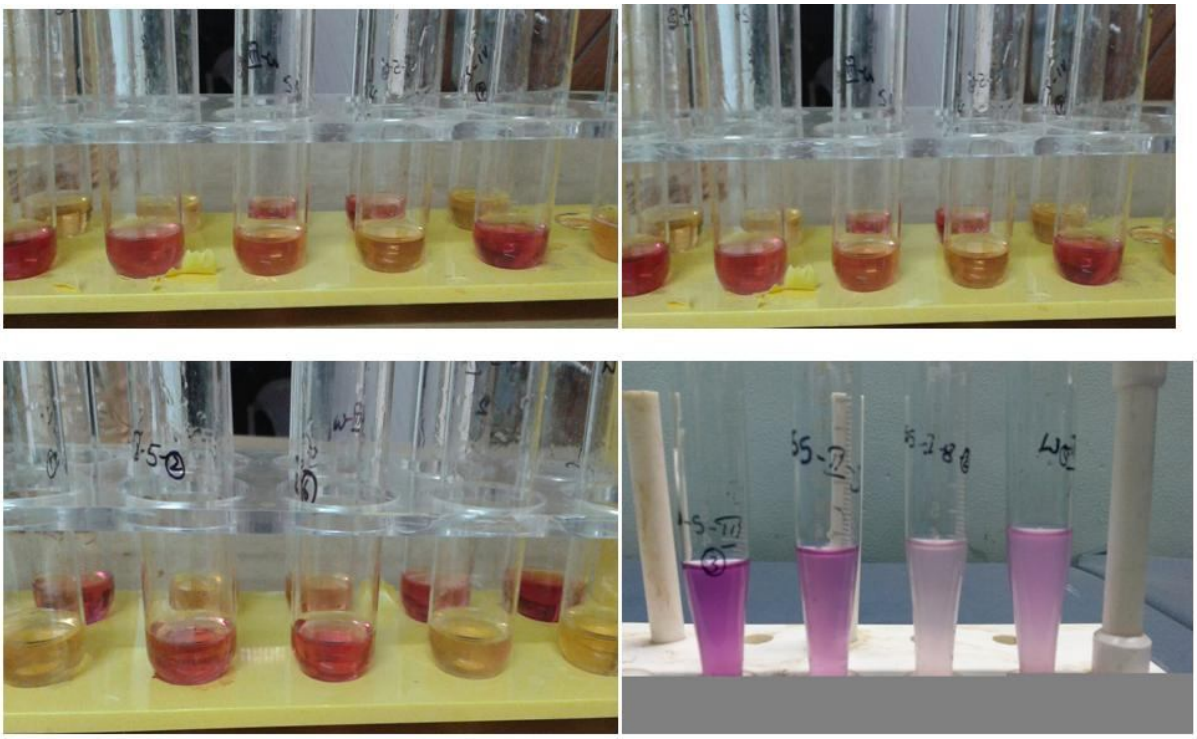
Figure C: Chromium reduction activity results



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