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HARNESSING NATIVE *PAENIBACILLUS LAUTUS* FROM INDUSTRIAL SOIL FOR SUSTAINABLE BIOREMEDIATION OF HEXAVALENT CHROMIUM

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ABSTRACT

Heavy metal pollution due to Industrial and mining activities remains a major environment challenge. Among various pollutants, hexavalent chromium [Cr(VI)]is highly toxic, soluble and carcinogenic. This study focused on bioremediation through isolation & screening of chromium-resistant bacteria from the mining soils of Hardi Bazar, Korba (C.G.) India. Minimum Inhibitory Concentration (MIC) method was applied to select the bacterial isolate with maximum tolerance against hexavalent chromium. The isolate which displayed maximum tolerance up to 900 ppm was sent for molecular identification via 16S rRNA gene sequencing using an Applied Bio systems 3730xl Genetic Analyser. The analysis confirmed that the strain belongs to the genus *Paenibacillus* with closest resemblance to *Paenibacillus lautus*. Further analysis using UV-Visible Spectrophotometry confirmed that this bacterium successfully reduced 24.17 % Cr(VI) from broth within 3 days of incubation .These results prove that *Paenibacillus lautus* has strong potential for cleaning up chromium-polluted soil .

KEYWORDS: Hexavalent Chromium, bioremediation, *Paenibacillus lautus*, heavy metals.

1. INTRODUCTION

Industrial growth and mining are responsible for environmental pollution, which is a significant issue worldwide (Barinova et al. 2020). Heavy metals are especially dangerous as they don't break down naturally, stay in the environment for months and can penetrate the food chain. Heavy-metals pollutants have caused severe deterioration of the environment due to their exclusive presence and high chemical reactivity(Agrawal et al. 2024;Alengebawy et

al.2021).Untreated industrial wastewater often contains metals like Pb, Cd, Hg, and Cr as a constituent (Bisht et al 2026). There are two primary classifications of chromium, which are trivalent [Cr (III)] and hexavalent [Cr (VI)]. Cr (III) is a micronutrient that is not toxic and essential for living organisms, but Cr (VI) has been identified as highly toxic, soluble, and capable of causing human cancer(Sharma et al 2020).It results in significant cell damage, protein synthesis and lasting DNA modifications (Xu et al. 2023; Zhigalenok et al.2025).

Conventionally different methods like ion –exchange, reverse osmosis, chemical filtration & membrane filtering have been utilized for removal of chromium from water.Inspite of their effectiveness, these techniques are expensive & require elaborate set ups(Peng et al.2020).Along with this they lead to generation of harmful chemical waste & are inefficient in removing low levels of metal contaminants. Researchers have thus focused on bioremediation as an alternative which is cost-effective & ecofriendly in nature. Application of biological systems like bacteria, fungi, algae and plants provide an excellent solution for breakdown and recycling of these pollutants. This approach is known as bioremediation. Employment of bacteria efficient in metal detoxification along with plant-beneficial characteristics is an economic and ecofriendly metal bioremediation approach (Ahemad et al.2019; Alidoosti et al. 2024; Karnwal et al.2024).Toxic environments are often accompanied by the development of resistance mechanisms by bacteria living in old industrial and mining areas.

Conversion of harmful hexavalent chromium Cr (VI) into safer trivalent chromium Cr (III) is facilitated by mechanisms like metal efflux pumps & cell surface binding special enzymes called chromate reductases (Bhunia et al.2022). Korba coal basin was chosen for isolation of metal resistant bacteria as it is an industrialized area known for mines & power plants (Shil et al.2025).

This study aims at screening and identification of chromium tolerant bacteria from the mining area of Hardi bazaar, Korba. Minimum inhibitory Concentration (MIC) analysis helped in selection of metal tolerant strain which was further applied for metal remediation studies through spectrophotometric analysis. It is designed to provide a reliable, localized bacterial strain for bioremediation of chromium-contaminated industrial sites.

2. MATERIAL& METHODS

Sampling site

The coal mining area of Chhattisgarh, India was used to collect soil samples. Sterile containers were used to collect surface soil from heavy-metal rich sites and brought to the laboratory for analysis. Microbiological analysis was conducted after storing samples at 4°C.

Figure 1: Sample Collection Area.

Chemical and media preparation

The chemical reagents and growth media employed in this study were of analytical grade. LB broth and Nutrient Agar media were prepared using standard procedure. A primary stock solution of hexavalent chromium (1000 ppm) was obtained by dissolving potassium dichromate ($K_2Cr_2O_7$) in deionized water. All microbial isolation, inoculation and sub-culturing procedures were performed under laminar airflow hood.

Isolation and screening of Bacteria

One gram of soil sample was vortexed with 9 milliliters of clean, sterile-distilled water to serially dilute the sample. Nutrient Agar plates containing $K_2Cr_2O_7$ were inoculated with 0.1 ml aliquots from these dilutions and then incubated at 37°C for 24 to 48 hours. Distinct colonies that emerged after incubation were picked and purified through frequent streaking on Luria-Bertani (LB) agar (Kalaimurugan et al. 2020).

Minimum Inhibitory Concentration (MIC) Assay

Broth dilution method was used to measure the chromium tolerance capacity of the isolated bacterial strains. Luria bertani broth tubes containing varying concentrations of potassium dichromate ($K_2Cr_2O_7$) i.e. 100 to 1000 ppm were prepared. Pure cultures of the bacterial isolates were inoculated aseptically & incubated in shaking incubator for 24-48 hours at 37°C and 120 rpm. Absence of growth of bacteria in a particular tube was visually observed in terms of turbidity. The concentration that totally inhibited bacterial growth was noted as Minimum Inhibitory Concentration (MIC) (Kookhaee et al. 2022).

Molecular Identification and Sequencing

Genomic DNA was extracted from the most tolerant strain. An Applied Biosystem 3730xl Genetic analyzer was used to sequence the amplified 16S r RNA gene using Polymerase Chain Reaction (PCR) and universal primers. Sequence Scanner Software v2.0 was employed to examine the raw electropherogram data (SampleID:CRS2-16SF_F09) and determined a reliable read length of 914 base pairs.

Chromium Reduction Analysis

The bacteria with highest MIC value was grown in LB broth with 500 ppm of potassium dichromate and then incubated for 3 days at 37°C before being centrifuged at 10,000rpm for 10 min to obtain cell-free supernatants. The remaining Cr (VI) concentration was measured using the 1, 5-diphenylcarbazide (DPC) method. The absorbance was measured using a UV-Visible spectrophotometer. Reduction efficiency was calculated using the standard decay formula (Lace et al. 2019).

3. RESULT AND DISCUSSION

Isolation and Screening of Chromium-Resistant Bacteria

A diverse population of bacterial colonies was observed in soil samples when serially inoculated on nutrient agar media containing hexavalent chromium [Cr (VI)]. Four morphologically different bacterial isolates were selected for further studies.

Evalution of Minimum Inhibitory Concentration (MIC)

All four isolates were subjected to increasing concentrations of potassium dichromate ($K_2Cr_2O_7$). Isolate-1 and isolate-2 didn't grow beyond 400ppm and 600ppm respectively. Isolate-3 displayed higher growth potential & probable strong metabolic defenses against heavy metal toxicity with a MIC value of 900ppm.

Table 1: Minimum Inhibitory Concentration (MIC) of Chromium-Tolerance Bacterial Isolates.

S.No	Bacterial isolate	Potassium dichromate concentration range(ppm)	MIC value(ppm)	Growth Observation	Chromium Tolerance
1	Isolate1	100-1000ppm	400ppm	Growth inhibited beyond 400ppm	Moderate
2	Isolate2	100-1000ppm	600ppm	Growth inhibited beyond 600ppm	High
3	Isolate3	100-1000ppm	900ppm	Growth observed up to900ppm	**Maximum tolerance**
4	Isolate4	100-1000ppm	500ppm	Growth inhibited 500ppm	Moderate to high

Molecular Identification and phylogenetic Analysis

The exact taxonomic identity of the most potent strain (Isolate-3) was analyzed through genomic profiling using 16S rRNA gene sequencing. The ABI 3730xl Genetic Analyser provided a high quality nucleotide sequence of 914 base pair(SampleIDCRS2-16SF_F09) with an excellent quality value score (QV20+: 883). The alignment under NCBI BLAST showed that the sequence was highly similar to that of *Paenibacillus* with closest strain being *Paenibacillus lautus*.

UV-Visible Spectrophotometric Analysis and Chromium Reduction Kinetics

The detoxification of Cr (VI) by *Paenibacillus lautus* was quantitatively assessed spectrophotometrically using the 1, 5-diphenylcarbazide (DPC) method. There was a clear decrease in the absorption peak of Cr (VI)- DPC complex after 3 days. The peak shifted from 428nm to 421 nm, a clear blue shift, suggesting there are alterations in the surrounding of the absorbing species. This may be attributed to formation of intermediate chromium species or the interference of other metabolites present. There is a possibility of complex formation between chromium and other biomolecules as well. The calculation of decrease in chromium percentage on the basis of change in absorbance values indicated that *Paenibacillus lautus* efficiently reduced chromium by 24.17 % in 3 days .This decrease suggests that the bacteria does not just absorb metals on its surface, but actively promotes the conversion of highly soluble Cr(VI) into safer and less solvable trivalent Cr(III) through enzymatic means.

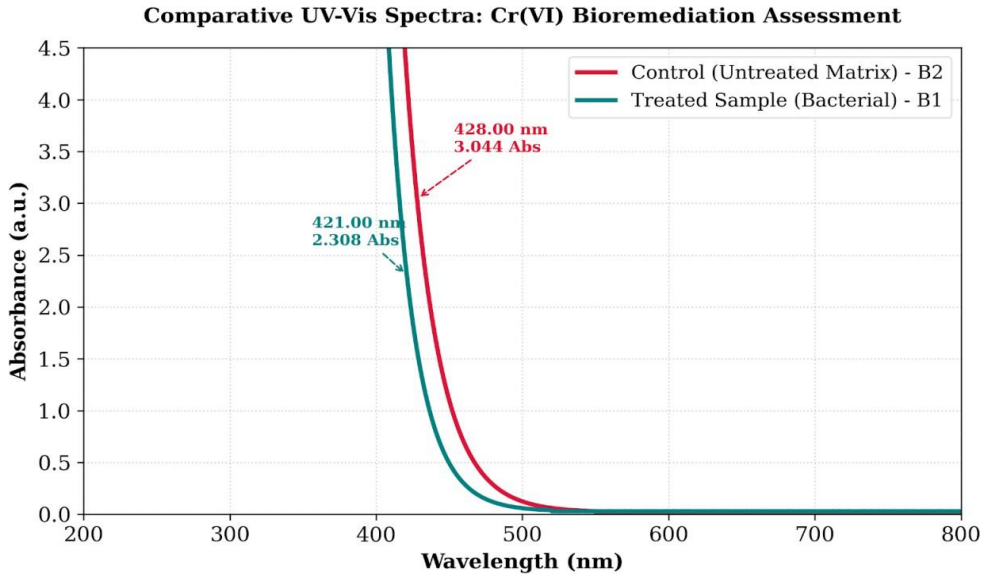

Figure 2: ComparativeUV-VisSpectra: Cr (VI) BioremediationAssessment.

B1-Treated sample

B2- Untreated Control

Formula for Chromium Bioremediation Efficiency%

$$\text{Bioremediation \%} = \frac{A_o - A_t \times 100}{Ao}$$

$$\text{Bioremediation \%} = \frac{3.044 - 2.308 \times 100}{3.044} = 24.17\%$$

Where A_0=Absorbance of Control (SampleB2=3.044)

A_t= Absorbance of Treated solution (Sample B1 = 2.308)

Table 3: UV-Visible Spectrophotometric Characterization Data.

Wavelength(nm)	Control B2 (Absorbance)	Sample B1 (Absorbance)
350	0.800	0.600
380	1.800	1.300
400	2.500	1.900
421	2.950	**2.308**
428	**3.044**	2.150
460	1.900	1.400
500	0.900	0.700
550	0.200	0.150

We mathematically determined the quantitative bioremediation efficiency of the chromium-resistant bacterial isolate by using the standard de-colorization and attenuation formula. By evaluating the initial absorbance of the untreated control matrix and the final absorbance of each bacterial culture within the designated incubation period, it was determined that the total bioremediation efficiency would be 24.17%. It can be concluded that the soil microbial isolate undergoes active metabolic processing and quantitative reduction, which leads to the transformation of hexavalent chromium.

4. CONCLUSION AND FUTURE SCOPE

The results of this study indicate that *Paenibacillus lautus* has the potential to detoxify chromium-stressed environments. Prior experiments have shown strains like *Bacillus* & *Pseudomonas* to effectively reduce chromate levels. The bacterium strain *Paenibacillus lautus* isolated in this study can be added to this category with its high MIC value of 900 ppm. It can be efficiently employed for bioremoval of Chromium and other heavy metals (Alotaibi et al.2021; Fakhar et al.2022).Previous researchers have demonstrated that *Paenibacillus* species display enhanced detoxification potential against various heavy metals like lead (Pb), cadmium (Cd) & chromium (Cr) due to cumulative actions like biosorption, bioaccumularion & enzymatic bioconversion (Khan et al. 2026; Belal et al. 2018; Harun et al.2024; Luo et al.2022). Further research can be carried out to analyze the effect of various physiochemical parameters like pH, temperature etc. on the bioremediation efficiency of this bacterial strain.Along with this genome sequencing studies can be carried out to identify presence of sequence coding "chromate reductase" (chr A OR yie F).Thus these preliminary results for *Paenibacillus* can lay the foundation of future in-situ bioremediation studies for ecofriendly and sustainable management of heavy metal pollution.

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