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ISOLATION OF CADMIUM-RESISTANT BACTERIA FROM BASSEIN CREEK AND THEIR POTENTIAL USE FOR BIOREMEDIATION OF HEAVY METALS

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Abstract: Cadmium (Cd), one of the heavy metals, is known to be a widespread environmental contaminant and potent toxin that can harm human health. Heavy metal pollution has become a major international issue. Using cadmium-resistant bacterial strains isolated from a Bassein creek water sample, this study set out to develop a practical strategy for attempting to eliminate cadmium from the environment.
A contaminated water sample from Vasai Creek, formerly Bassein Creek, an estuary and one of the two main Ulhas River distributaries in the Konkan division of Maharashtra, India, was used to isolate eight cadmium-resistant bacterial strains for this study. Two of these isolates, designated C1 and C6, were found to be tolerant of cadmium contamination and to thrive in the laboratory. The minimal inhibitory concentration, which ranged from 50 to 1000 ppm, was also used to screen these bacterial isolates for metal resistance to Cd^{2+}. C1 was found to be tolerant of Cadmium levels up to 200 ppm, while C6 was found to be tolerant of Cadmium levels up to 400 ppm. In addition, a study using an atomic absorption spectrophotometer (AAS) revealed that both strains (C1 and C6) were able to significantly absorb and remove 96.15% and 96.08% of Cadmium within five days, respectively. The obtained isolates may be Micrococcus or Arthrobacter sp., according to the biochemical tests. As a consequence of this, the study demonstrated that the bacterial strains C1 and C6 can be actively utilized for the bioremediation of heavy metals like cadmium, a significant toxic pollutant that is present in industrial events.
Chemical precipitation, solvent recovery, and other conventional approaches have a number of drawbacks, including the production of toxic sludge and the unpredictable removal of metal ions. Therefore, the removal or detoxification of heavy metals primarily from soil, water, and sediments can be effectively accomplished through microbial remediation using these two strains of bacteria.

Keywords: Bioremediation, Cadmium, heavy metals, Biosorption, Bacteria, Eco-friendly, Pollution, Environment, AAS, water, Micrococcus, Arthrobacter, metal resistance

I. INTRODUCTION

All living beings' health is at risk from heavy metal bioaccumulation in the natural environment. The quality of water and the things that depend on it is impacted by water pollution brought on by industrial effluents that contain toxic sludge, heavy metals, and a variety of solvents. Animals, plants, humans, and aquatic biotopes all pose health risks when heavy metals from industrial waste enter aquatic ecosystems. Mercury (Hg), copper (Cu), chromium (Cr), zinc (Zn), cadmium (Cd), and lead (Pb) are heavy metals that cause mutations, are toxic to cells, and can cause cancer in humans and other organisms. Likewise, untreated or to some extent treated modern wastewater released with heavy metals into water bodies may fundamentally influence the groundwater too. Heavy metal pollution can be remediated by microbes in the environment [Pushkar et al., 2019]. To break down the complex wastes, the microbes create a variety of metabolites and learn how to survive in the presence of a variety of toxic heavy metals in their environment. Even at very low concentrations, cadmium (Cd), one of the major pollutants, is extremely toxic to organisms. Cadmium is chiefly utilized in different businesses including paint, copper alloy, mining, alkaline batteries, paper and pulp, zinc refining, and manure.

Through the food web, cadmium bioaccumulates and can cause a variety of serious diseases in animals and humans. Cd inhibits DNA-mediated transformation in microorganisms, their cellular enzyme functions, and the symbiotic relationship between plants and microbes, but it is not required for any biological function. In addition, the majority of plants' bioaccumulation of Cd may disrupt a variety of biochemical functions, structural changes, and physiological processes. These functions include altering the function of photosynthesis and mineral uptake, interfering with enzymes involved in the Calvin cycle and the metabolism of carbohydrates, lowering crop productivity, and altering antioxidant metabolism in plants. Heavy metals are removed from the aquatic environment using a variety of methods. The normal techniques incorporate compound oxidation, substance precipitation, decrease, filtration, electrochemical treatment, and extraction utilizing solvents.

The unpredictability of the removal of heavy metals and the large amount of highly toxic sludge generated by these traditional methods are two of the many disadvantages. Bioremediation, which uses recombinant and naturally occurring indigenous microorganisms to remove heavy metals effectively, is an alternative strategy. Bioremediation is less expensive than chemical methods and is better for the environment. Additionally, the bioaccumulation and biosorption processes are utilized to remove metals from the dead biomass of living or dead bacteria. Energy is required for the oxygen-dependent bioaccumulation process. Biosorption, on the other hand, is a revisable independent process that does not call for energy or respiration. This method has a significant advantage due to its high sorption capacity, low operating costs, potent biosorbent revival, and metal recovery potential [Al-Dhabi et al., 2019].

In this investigation, a water sample from Bassein Creek that contained Cd pollutants was used to isolate a potent Cd-resistant strain C1 and C6. They were found to be Micrococcus and Arthrobacter species respectively based on biochemical characterization. The degree of tolerance for cadmium and the likelihood of its elimination by these strains were examined. Additionally, the isolated bacterial strains' capacity to remove Cd raises new prospects for its use as a Cd bioremediating agent.

II. MATERIALS AND METHODS

2.1 Collection of Sample:

The Water sample was collected from Bassein creek, specifically where industrial effluents are discharged in sterile 50-ml screw cap glass bottles covered with silver foil and stored at 4 °C till further analysis.

2.2 Enrichment and Isolation of cadmium-resistant bacteria:

10 ml of water sample was inoculated in 90 ml of sterile Nutrient broth supplemented with 50, 100, and 150ppm of cadmium distinctly and incubated at room temperature under static conditions for 5 days. After Enrichment, a loopful from the broth was taken for isolation of cadmium-resistant bacteria from Bassein creek. cadmium-resistant bacteria were isolated on nutrient agar medium containing 50, 100, and 150 ppm (Parts per million) of cadmium, respectively using the spread-plate method. Nutrient agar plates were incubated at 37°C for 24 hours. After the incubation period, morphologically different colonies were selected from nutrient agar plates for further study. Isolated bacterial colonies selected from nutrient agar plates were further inoculated in nutrient broth and incubated at 37°C on a shaker incubator with 150 rpm (revolutions per minute) for 24 hours. After the incubation period of 24 hours, a loop full of bacterial culture from the nutrient broth was further subjected to isolation on a nutrient agar plate and incubated at 37 °C for 24 h. The above procedure was repeated 2–3 times to get the purified isolated bacterial colonies comprising a single

type of bacteria. The Purity of bacterial colonies was confirmed by the Gram staining technique. Purified bacterial isolates were sub cultured till further study.

2.3 Study of Colony Characteristics of the isolated Cadmium resistant bacterial strains:

The isolated colonies were examined with respect to their visual appearance on the agar plate such as shape, edge, color, optical properties, texture, etc.

2.4 Evaluation of Minimum Inhibitory concentration of Cadmium by plate method:

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of the heavy metal at which no visible growth of microorganisms is observed after an incubation period of 24 hours. In the current investigation, the MIC of the cadmium-resistant bacterial isolate was determined by using a nutrient agar medium containing cadmium in a range of 50 ppm to 1000 ppm. Log phase culture of cadmium-resistant bacterial isolates was isolated on the above nutrient agar medium and incubated at 37°C for 24 hours. After the incubation period of 24 hours, nutrient agar plates were observed for the presence and absence of bacterial growth.

2.5 Cadmium Accumulation and Removal Assay:

The selected bacterial strains were cultured in the Nutrient Broth (NB) containing Cd (100 ppm). The culture was incubated on a rotary shaker at 37°C for 5 days. The culture medium without Cd was used as the negative control. The centrifuged cell-free supernatant was stored at 4°C for the analysis of Cd remediation. The Cd content of the cell-free supernatant was detected using atomic absorption spectrometry with a Cd hollow cathode lamp at 228.8 nm. The percentage of cadmium removal by the bacterial cells from the culture was calculated by taking the difference between the initial metal content in the culture media and at the time of determination of the same.

2.6 Biochemical Characterization

Cadmium-resistant bacteria isolated in the current study were analyzed for their biochemical characteristics. Bacterial isolates were analyzed for their ability to ferment glucose, lactose, maltose, mannitol, and sucrose. Bacterial isolates were also tested for indole, methyl red, Voges–Proskauer, and Simmons citrate tests (IMViC test). Other biochemical tests included in the study were motility, gelatinase, and catalase. Biochemical characterization of bacterial isolates helped in determining the genus of cadmium-resistant bacterial isolates

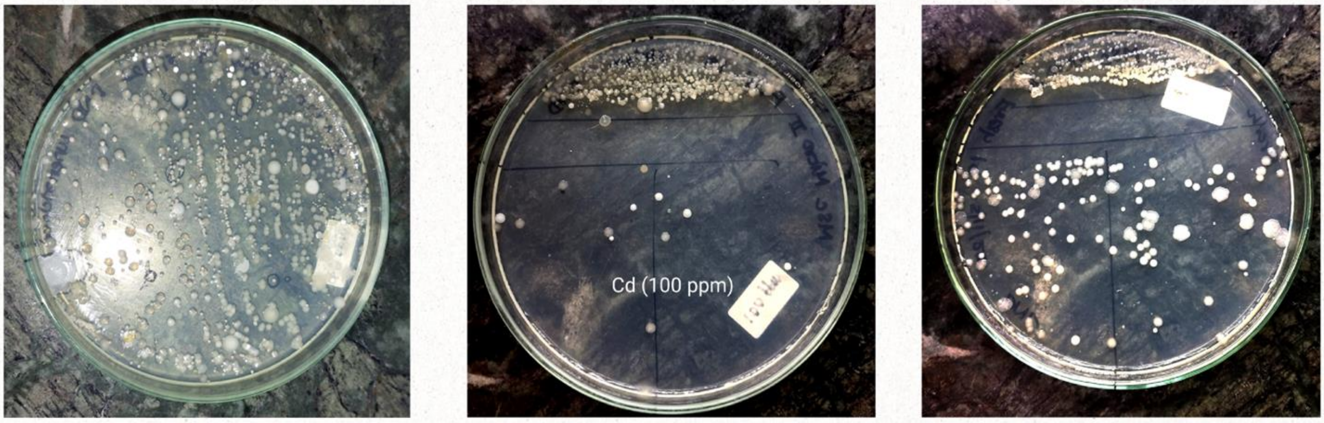
2.7 Identification of the Bacterial strains:

Identification of the isolates was done by employing Bergey's manual based on the results obtained from Biochemical characterization.

III. RESULTS AND DISCUSSION

3.1 Isolation of Cadmium-resistant Bacteria:

After the enrichment of the water sample, isolation was carried out on Nutrient agar supplemented with 50, 100, and 150 ppm of Cadmium respectively. The obtained colonies were sub-cultured and a total of 8 bacterial isolates were obtained which were selected and then maintained on slants for further tests.



A) Bacterial colonies on Nutrient Agar supplemented with 50 ppm of Cadmium

B) Bacterial colonies on Nutrient Agar supplemented with 100 ppm of Cadmium

C) Bacterial colonies on Nutrient Agar supplemented with 150 ppm of Cadmium

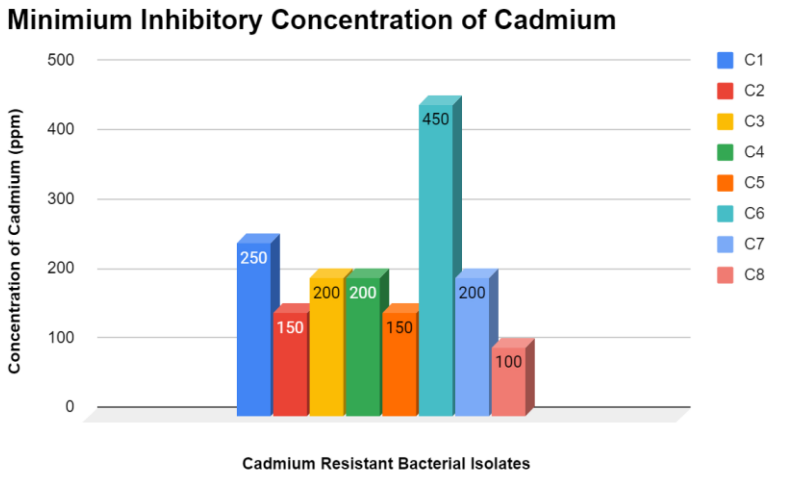
Figure 1: Isolation of Cadmium-resistant bacterial strains on Nutrient Agar supplemented with different concentrations of cadmium

3.2 Study of Colony Characteristics of the isolates:

Table1: Colony Characteristics of all the selected isolates

Characteristics	Colonies on NA supplemented with 50 ppm of Cd			Colonies on NA supplemented with 100 ppm of Cd		Colonies on NA supplemented with 150 ppm of Cd		
	C1	C2	C3	C4	C5	C6	C7	C8
Size	<1mm	< 1mm	> 2mm	<1mm	>3mm	<1mm	<1mm	>2mm
Color	Slight Orange	Yellow	Beige	Beige	Slight orange	Beige	Slight orange	Beige
Shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Irregular
Margin	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Elevation	convex	raised	convex	convex	convex	convex	convex	raised
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Consistency	Butryous	Butyrous	Mucoid	Mucoid	Butyrous	Mucoid	Butyrous	Brittle (dry)
Gram Character	Gram +ve	Gram +ve	Gram +ve	Gram +ve	Gram -ve	Gram +ve	Gram -ve	Gram +ve
Arrangement	Cocci in clusters	Cocci in tetrads and clusters	Short rods in singles and cocci in tetrads and clusters	Short rods in singles and cocci in singles and clusters	Short rods in singles and cocci in clusters	Cocci in clusters	Cocci in clusters	Short rods in singles and cocci in tetrads and clusters

3.3 Evaluation of Minimum Inhibitory Concentration (MIC) of Cadmium:



Graph 1. MIC of all the 8 isolates (C1, C2, C3, C4, C5, C6, C7 and C8)

Figure 2. MIC of isolate C1 - 250 ppm

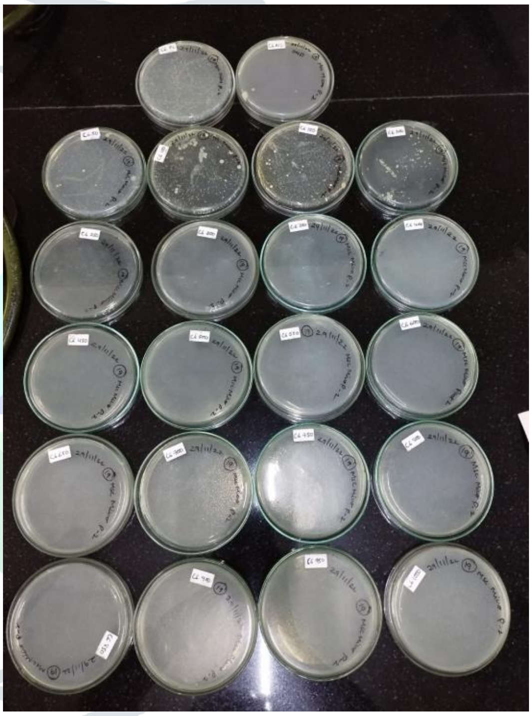


Figure 3: MIC of isolate C6 - 450 ppm

3.4 Cadmium Accumulation and Removal Assay:

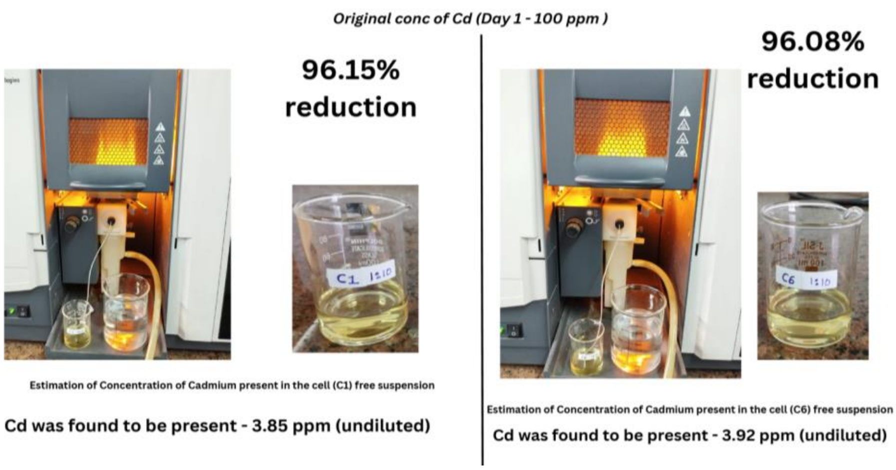
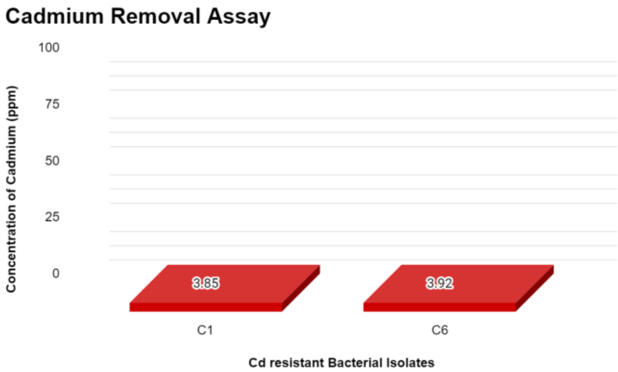
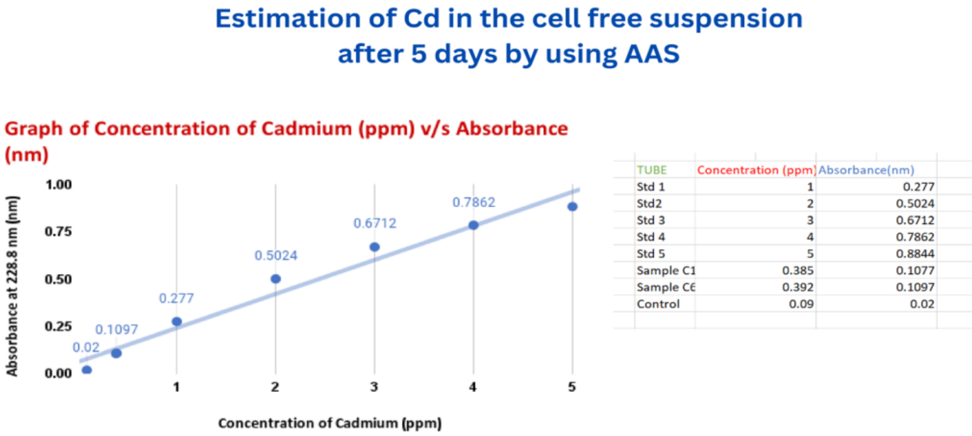


Figure 4: Estimation of Cadmium in cell-free supernatant by Atomic Absorption Spectroscopy after 5 days of incubation





TUBE	Concentration (ppm)	Absorbance(nm)
Std 1	1	0.277
Std2	2	0.5024
Std 3	3	0.6712
Std 4	4	0.7862
Std 5	5	0.8844
Sample C1	0.385	0.1077
Sample C6	0.392	0.1097
Control	0.09	0.02

Graph 3: Concentration of cadmium (ppm) versus Absorbance at 228.8nm

3.5 Identification of the isolates: (Later these results were compared with Bergey's manual)

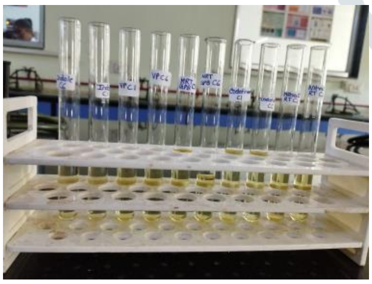


Figure 5: Biochemical media (Before Incubation)

Figure 6: Biochemical media (After Incubation)

Figure 7: Biochemical media (After Incubation)

Figure 8: Sugar Fermentation test (Media before incubation)

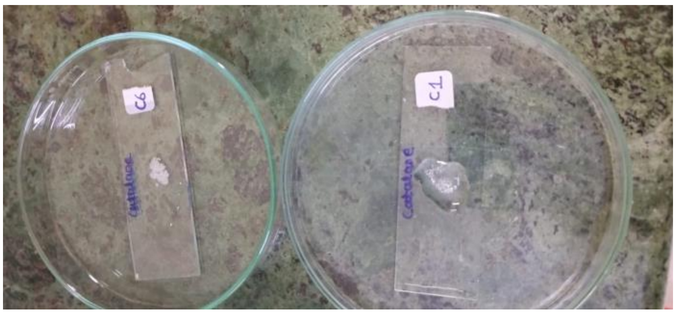


Figure 9: Positive Catalase test for both C1 and C6 isolate

Table 2: Biochemical characterization of the potential isolates (C1 and C6)

Biochemical Tests	Colony 1 (C1)	Colony 6 (C6)
Catalase	Positive	Positive
Indole	Negative	Negative
Vogues Proskauer	Negative	Negative
Citrate Utilization	Positive	Negative
Gelatinase	Positive	Positive
Nitrate Reduction	Positive	Negative
Methyl Red Test	Positive	Negative
Phenylalanine deaminase	Negative	Negative
Glucose Fermentation	Positive	Negative
Lactose Fermentation	Negative	Negative
Mannitol Fermentation	Negative	Negative
Maltose Fermentation	Positive	Negative
Sucrose Fermentation	Positive	Negative

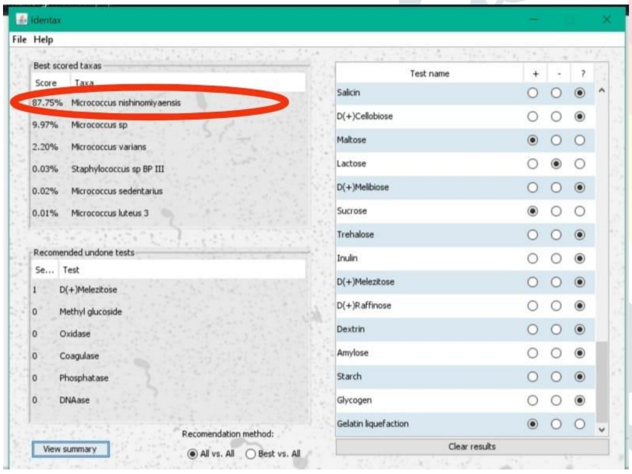
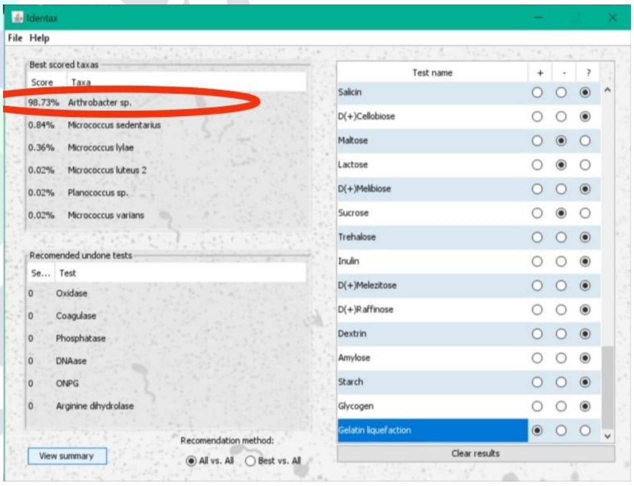


Figure 10: Isolate C1 was found to be Micrococcus spp. (Comparison with Bergey's Manual using IDENTAX - an online software) based on the results obtained from biochemical tests



(comparison with Bergey's Manual using IDENTAX - an online software) based on the results obtained from biochemical tests

IV. CONCLUSION:

The capacity of the chosen bacterial strains to thrive in the presence of Cd would be extremely valuable in wastewater treatment or harmful xenobiotic component bioremediation. Micrococcus and Arthrobacter species isolated from a metal-contaminated environment were shown to be extremely resistant to Cd and grew well at exposure levels of 200 ppm and 400 ppm, respectively. Both identified isolates demonstrated a high level of resistance to Cadmium and were able to remove up to 96% of cadmium in five days, implying that these strains can be used efficiently for bioremediation and removal of cadmium present in polluted water, soil, and sediments at a low cost and with high efficiency.

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