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Exploring the Bioremediation Potential of Halophilic Bacteria on Heavy Metal Pollution

Kiruthika. P[1], Karthik Sundaram. S[2]

[1]M.Sc Microbiology, Dr. N.G.P Arts and Science College. Coimbatore-641048
[2]Associate professor, Department of Microbiology, Dr. N.G.P Arts and Science College, Coimbatore-641048

ABSTRACT

Heavy metal pollution is the major cause of detrimental environmental pollution and human health. This is especially observed in saline habitats where conventional bioremediation methods are insufficient. This study investigates halophilic organisms' capacity to heal heavy metal contamination. The samples for halophilic organism recovery were isolated from the Thrissur and the heavy metal samples were prepared in different concentrations (100ppm, 200ppm, 500ppm, and 1000ppm). The isolated samples were incubated with the SGYE medium and appropriate colonies were separated, The isolate was analyzed for the heavy metal remediating properties by analyzing the growth of the halophilic organism in the presence of heavy metals and screening whether it can grow in different concentrations of heavy metals. Then the isolated organism tends to nucleic acid configuration for the profiling by MALDI-TOF. The strain resists various metals, and the growth pattern is studied using Atomic absorption spectroscopy (AAS). The investigation indicated that the organism can degrade the metals in the high percentile.

Keywords: Bioremediation, halophiles, heavy metals, detoxification, and resistance.

INTRODUCTION

Heavy metals exhibit persistence, bioaccumulation, and toxicity characteristics that threaten environmental quality and human health. Heavy metals can accumulate in sediments, aquatic plants, and organisms in aquatic environments, disrupting food chains and biodiversity. Additionally, heavy metals can leach into groundwater, contaminating drinking water sources and posing risks to human health. In terrestrial ecosystems, heavy metal contamination can lead to soil degradation, reduced crop yields, and bioaccumulation in plants, posing risks to both human and animal health through consumption (Zhang *et al*., 2017). There are various health Implications of Heavy Metal Exposure, the exposure to heavy metals can have severe health consequences for humans, ranging from acute poisoning to chronic health effects. endowed with unique physicochemical properties characterized by high concentrations of dissolved salts, alkalinity,and metal ions, provide a fertile breeding ground for halophiles—microbial pioneers of the salty frontier (Oren, 2013). However, the idyllic tranquility of saline habitats is shattered by the relentless onslaught of human activities, as industrial discharges, urban sprawl, and agricultural runoff conspire to disrupt the fragile equilibrium, unleashing a torrent of heavy metal contaminants into the pristine waters (Mal *et al*., 2019). Moreover, heavy metals can bioaccumulate in the food chain, leading to long-term health risks for populations dependent on contaminated food and water sources. In response to the growing concerns about heavy metal

pollution, regulatory agencies have implemented measures to monitor and control the release of heavy metals into the environment. These regulations include emission standards, pollution prevention guidelines, and remediation requirements to reduce heavy metal contamination and protect environmental quality. Remediation strategies for heavy metal pollution include physical methods such as excavation and containment, chemical treatments such as precipitation and ion exchange, and biological approaches such as phytoremediation and microbial bioremediation. The Promise of Halophiles in Bioremediation is amidst the desolation wrought by heavy metalpollution, a glimmer of hope emerges on the horizon in the form of halophiles—nature's unsungheroes, resilient denizens of the salty abyss (Das & Chandran, 2011).

MATERIALS AND METHODS

Collection of samples

The saline soil and saline water samples were collected from the valappad beach, chavakkad, Thrissur, Kerala. These samples are collected to isolate halophiles (salt-loving organisms). Collected samples are then transferred into a polythene zip lock cover and transferred to the laboratory.

BACTERIAL ISOLATION AND IDENTIFICATION

Enrichment of sample

Bacteria are isolated from the collected saline sample. 2g of soil sample and 2 ml of water sample were weighed in a beaker and mixed with 100 ml distilled and filtered through the Whatman filter paper. After filtration 100 µL solution was added to a 25% NaCl-containing liquid medium and incubated at 37°C. SGYE media is used for the cultivation of halophilic microorganisms. After the formulation of the medium, the pH of the medium is adjusted to the range of 7 which isdesirable for the growth of halophiles using 1 M NaOH solution. The medium is then autoclaved at 121°C for 20 minutes for the elimination ofmicroorganisms.

Isolation of bacteria

After the enrichment of the soil sample, the serial dilution technique is performed to isolate the pure culture of bacteria. 1 ml of the soil sample is added to 9 ml of Dilution Blank Tube. This is then followed by the same procedure, where 1 ml from Tube 1 is added to 9 ml of Tube 2, 1 ml from Tube 2 is added to 9 ml from Tube 3, and so on until the desired concentration is reached. 1ml of diluted samples were transferred into Starch glucose yeast extract agar plates and incubated at 37°C for 7 days.

MICROSCOPIC EXAMINATION

Gram Staining Technique

One loopful of culture was taken and spread on a clean glass slide. And allowed the smear to air dry and then heat fixed flooded the smear with crystal violet and waited for 1 minute. Wash the slide in a gentle and indirect stream of tap water for 2 seconds. Flood slide with the mordant: Gram's iodine. Wait 1 minute. Wash the slide in a gentle and indirect stream of tap water for 2 seconds. Flood slide with a decolorizing agent. Wait 15 seconds or add drop by drop to slide until the decolorizing agent running from the slide runs clear. Flood slide with a counterstain, and safranin. Wait 30 seconds to 1 minute. Wash slide in a gentile and indirect stream of tap water until no color appears in the effluent and then blot dry with absorbent paper. Observe the results of the staining procedure under oil immersion using a Brightfield microscope.

BACTERIAL CONFIRMATION TEST

VITEK MS PRIME is a benchtop, high-throughput, automated microbial identification system that uses a mass spectrometry system using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) for the identification of microorganism's culture. (Sarkar *et al.*2019)

EVALUATION OF BIOREMEDIATION POTENTIAL OF HALOPHILE

Stock solutions of Cadmium, Iron, Barium, and Copper (1000 mg/L) were prepared from corresponding metal salts (i.e. $CdCl_2$, $FeSo_4.7H_2O$, $BaCl_2.2H_2O$, $CuSO_4.5H_20$). The glassware used for this purpose was leached in 2N HNO_3 and rinsed several times with distilled water before use to avoid metal contamination. Fe^{2+} is oxidized to Fe^{3+} in the presence of nitric acid. 1ltrs of a stock solution of each metal ion was prepared in distilled water and acidified with HNO_3 (10-20 ml of 2% HNO_3) to prevent precipitation and was sterilized at 121°C for 15 min.

SAMPLE PREPARATION FOR METAL ABSORPTION

Various concentrations of heavy metals i.e. 100-1000 (mg/L) were prepared in a final volume of 10 ml in Starch Glucose Yeast Extract (SGYE) broth, and 1 ml of 24 hours isolated bacterial cultures were inoculated at 37°C for 3 days.

DETERMINATION OF METAL UPTAKE BY *Halomonas elongata*

Atomic Absorption Spectroscopy (AAS):

Atomic Absorption Spectroscopy (AAS) was employed to quantify the concentrations of heavy metals (barium, copper, cadmium, and ferrous) in samples before and after bioremediation treatments. The AAS analysis was conducted using a highly equipped analyzer. Calibration standards for each metal were prepared by diluting commercially available standard solutions to concentrations of 100 ppm, 200 ppm, 500 ppm, and 1000 ppm**.**

RESULTS

BACTERIAL ISOLATION AND IDENTIFICATION

Enrichment of bacteria

The enrichment of the sample in 25% NaCl containing liquid medium and incubated for 7 days at 37°C.

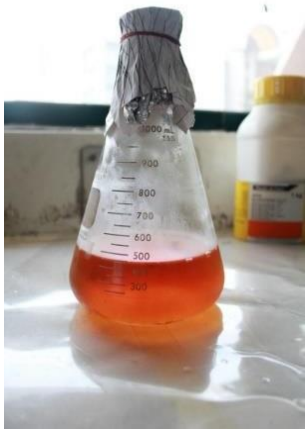


Figure 1: Enrichment of halophiles with 25% NaCl

Isolation of Bacteria

After the enrichment of the collected sample, the serial dilution technique is performed to isolate the pure culture of bacteria. 1 ml of diluted samples were transferred into SGYE agar plates and incubated at 37°C for 7 days. After the incubation period, a growth of white mucoid colonies was indicated on an SGYE agar plate.

Figure 2: Isolated halophile colonies

MICROSCOPIC OBSERVATION

Gram staining

In the gram-staining method, gram-negative, rod-shaped bacteria were seen

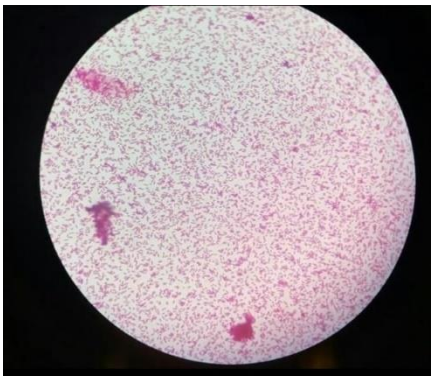


Figure 3: Microscopic view of isolated bacteria

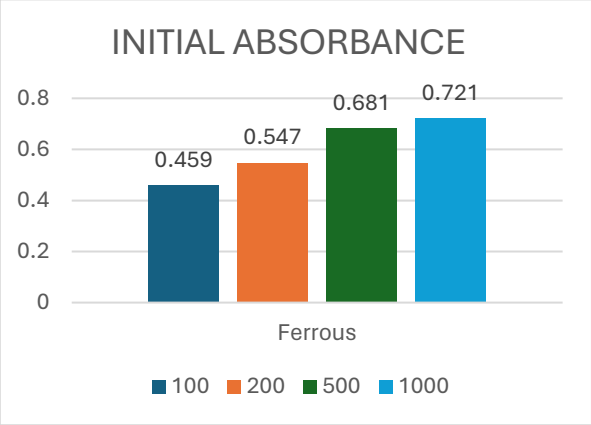
BACTERIAL CONFIRMATION TEST

The culture was identified in the Royal Care Hospital by the VITEK® MS PRIME method. The culture was identified as a ***Halomonas elongata.*** The organism identified shows 99.9% Probability in VITEK MS PRIME (which is a mass spectrometry system using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) for the identification of microorganism's culture).

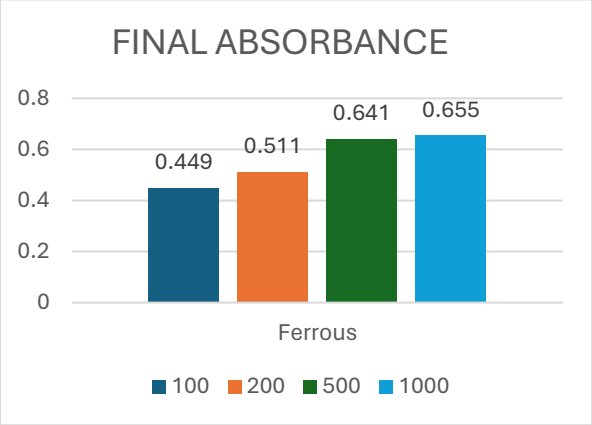
Among the microbial species identified through MALDI-TOF MS analysis, *Halomonas elongata* was consistently detected in multiple samples collected from varying depths (0-10 cm, 10-20 cm, and 20-30 cm). The mass spectra obtained from these samples exhibited characteristic peaks correspondingto the protein profiles of *Halomonas elongata*, as confirmed by comparison with reference spectra in the MALDI-TOF MS database.

ATOMIC ABSORPTION SPECTROSCOPY RESULTS

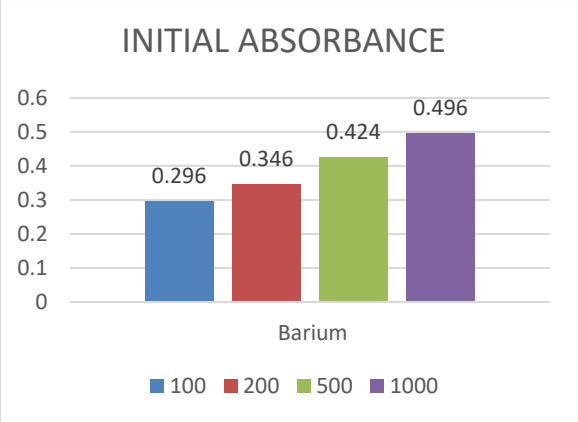
The heavy metals (Barium, Copper, Cadmium, and Ferrous) were treated with halophilic microorganisms to determine the degrading capacity of halophiles towards the heavy metals. Heavy metals were taken for Atomic absorption spectroscopy before and after the bioremediation forcomparative studies.



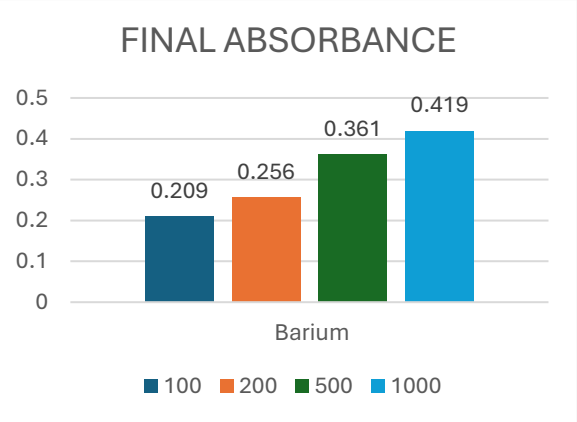
(a) Initial absorbance of Ferrous



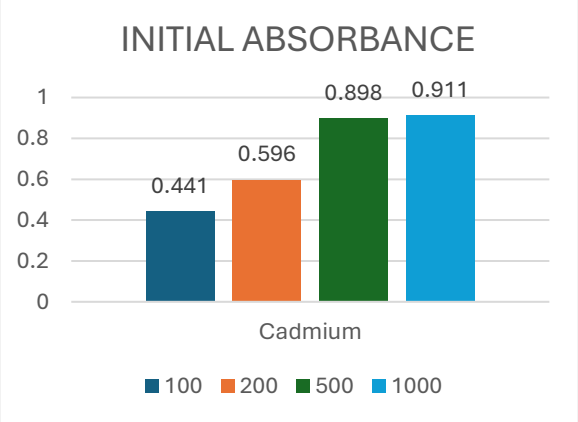
(b) Final absorbance of Ferrous



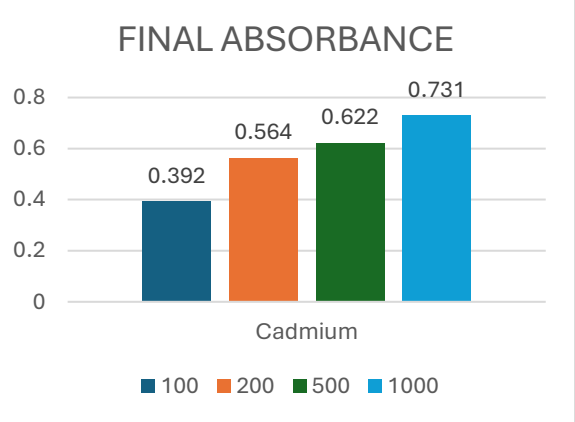
(c) Initial absorbance of Barium



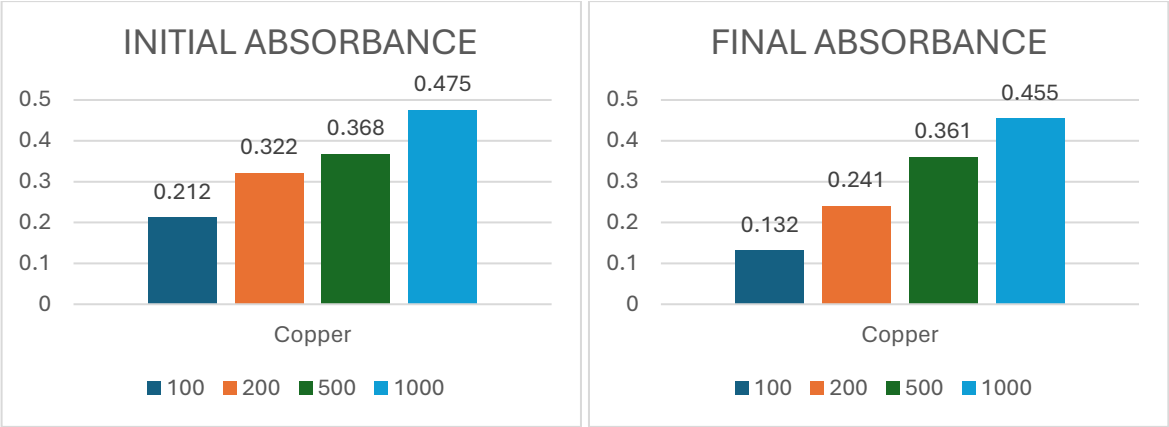
(d) Final absorbance of Barium



(e) Initial absorbance of Cadmium



(f) Final absorbance of Cadmium



(g) Initial absorbance of Copper **(h) Final absorbance of Copper.**

Figure 9: Initial and final absorbance of heavy metals.

The results of the AAS analysis revealed a significant reduction in the concentrations of heavy metals in the samples following bioremediation treatment. Specifically, there was a decrease in the levels of **Cadmium< Barium < Ferrous < Copper** ions, indicating the efficacy of the bioremediation processin mitigating heavy metal contamination in the soil.

CONCLUSION

This study involves the isolation of halophilic organisms from the saline environment and exploring their heavy metal degrading properties and their application in aquatic environments. The findings from the experiment can be summarized as follows: Isolation and identification of halophilic organism, *Halomonas elongate* was isolated from the saline sample collected from the valappad beach, Thrissur, Kerala. Soil samples were enriched and subjected to serial dilution for isolation of pure bacterial cultures, which were then analyzed using Gram staining and the VITEK MS PRIME microbial identification system. Stock solutions of heavy metals (Cadmium, Iron, Barium, Copper) are prepared and sterilized for subsequent experiments. The metal uptake by *Halomonas elongata* is investigated by exposing bacterial cultures to various concentrations of heavy metals and analyzing metal concentrations before and after bioremediation using atomic absorption spectroscopy (AAS). The AAS analysis helps in the identification of the growth pattern of the organism in the various concentrations of heavy metal.

REFERENCE

1. Oren, A. (2013). Life at high salt concentrations, intracellular KCl concentrations, and acidic proteomes. Frontiers in microbiology, 4, 315.
2. Mal, J., Nandi, R., Bera, P., Bhattacharya, M., & Chatterjee, A. (2019). An insight into halophiles: biotechnological implications and future perspectives. Journal of Basic Microbiology, 59(6), 535-556.
3. Das, N., & Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: An overview. Biotechnology Research International, 2011, 941810.
4. Sarkar, A., Kazy, S. K., & Sar, P. (2019). Characterization of bacteria from the family *Halomonadaceae* and *Virgibacillaceae* for bioremediation of heavy metals from a contaminated environment. Journal of Environmental Management, 232, 891-902.
5. Zhang, C., Liu, L., Su, H., & Pan, Y. (2017). Bioleaching of heavy metals from sewage sludgeby indigenous moderate thermophiles: microbial community analysis and temperature influence.

Bioresource Technology, 245, 1094-1102.
6. Pacyna, J. M., *et al*. (2007). Global emission of mercury to the atmosphere from anthropogenic sources in 2005 and projections to 2020. Atmospheric Environment, 41(37), 7656-7669.
7. Qureshi, A. S., *et al*. (2019). Advances in analytical techniques for arsenic speciation analysis. Trends in Analytical Chemistry, 118, 685-698.