Original Article
ISSN (Online): 2582-7472

ShodhKosh: Journal of Visual and Performing Arts
June 2024 5(6), 640–646

ISOLATION AND SCREENING OF LEAD-TOLERANT BACTERIA FROM CEMENT CONTAMINATED SOIL FOR POTENTIAL BIOREMEDIATION APPLICATIONS

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DOI
10.29121/shodhkosh.v5.i6.2024.1838

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

ABSTRACT

Bioremediation of metallic pollutants using heavy metal-tolerant bacteria is crucial to environmental biotechnology. This biological process involves the removal of heavy metals from contaminated areas. The first step in bioremediation is the screening of metal-tolerant bacteria.

In this study, the soil contamination level of a cement plant was investigated by measuring the concentration of certain heavy metals. This study aimed to screen lead-tolerant bacterial strains from contaminated cement soil for use in bioremediation. Sixteen lead-tolerant strains were isolated from the soil of the two cement plant areas. The strains exhibited varying levels of tolerance to lead, with minimal inhibitory concentrations (MIC) ranging from 15 to 33 mg L-1. The most tolerant strains were selected for further research to assess their potential for bioremediation.

The metal tolerance levels of a bacterial community isolated from a cement dust-polluted soil environment were assessed using different methods, including agar dilution, gradient plate, and MIC. The results showed that the bacteria were able to tolerate high concentrations of lead and that the MIC method was the most effective in evaluating their tolerance.

For further studies, most tolerant isolates were selected with maximum MIC values, including BCN-B1/5/Pb and BCN-B1/9/Pb from the BCN-B1 sample and PCN-P1/1/Pb from the PCN-P1 sample. These isolates demonstrated high tolerance to lead, with MIC values of 33, 33, and 31 mg L-1, respectively.

Keywords: Bioremediation, Cement Dust Pollution, Heavy Metal, Soil Bacteria, Lead-Tolerant Strains, MIC

1. INTRODUCTION

Heavy metal pollution is a pressing global environmental concern due to its detrimental effects on the environment and human health. The presence of toxic metals in soil can alter its physical and chemical properties, restricting plant growth and potentially entering the food chain (Maphuhla et. al., 2021). Bacteria that demonstrate the capacity to survive in toxic heavy metal concentrations have been isolated from different sources (Basu et al., 1997; Silva et al., 2003; Choudhury & Kumar, 1998; Duxbury, 1986; Haefeli et al., 1984; Lima-Bittencourt et al., 2007; Otth et al., 2005).

In the environment, metals may select these resistant variants assuming that the selective pressure exerted by heavy metals. Heavy metals' harmful impacts on microbes are affected by many variables, including pH and the concentration of organic material, speciation, and chelating agents (Duxbury, 1986; Nwuche and Ugoji 2008; Serrit and Lester, 1980)

Nevertheless, it has been shown that heavy metal-resistant bacteria accumulate a lot of metal ions capacity. Several of them have been used successfully in contaminated site remediation due to their capabilities elsewhere in the developed world (Owolabi and Hekeu, 2015). Bioremediation is effective in removing heavy metals from contaminated soil (Nanda et al., 2011). One approach is to use microorganisms that can extract metals from the environment. This process involves screening for microorganisms that can tolerate high concentrations of heavy metals and then using these microorganisms to clean up contaminated soil (Yakoubi et.al., 2017).

Satna is a city where population growth, urbanization, and industrialization have outpaced efforts to control pollution and environmental degradation. The effluent discharge from cement industries, health establishments, research centers, and mineral deposits has led to the contamination of the environment with toxic heavy metals, including lead. Lead is a particularly hazardous metal due to its toxic properties and non-biodegradability. Lead is a highly toxic metal that can have devastating effects on human health, particularly in young people. Exposure to lead can lead to mental retardation and other serious health issues. Lead is one of the main pollutants in soil, water, and air, and it is extremely harmful to humans, animals, plants, and bacteria. As hazardous waste, it poses significant environmental and health risks. One effective way to eliminate lead from the environment is through bioremediation, a process that uses bacteria to render lead non-bioavailable.

This current investigation aimed to examine the ability of lead-tolerant bacteria and their potential to bioremediate lead-contaminated cement dust soil. The use of metal-resistant microorganisms could also benefit the bioremediation of sewage and wastewater, as well as soil contaminated with heavy metals.

2. METHODOLOGY

In this study, we focused on three strains of lead-tolerant bacteria that were isolated from cement-contaminated soil. We evaluated the ability of these bacteria to tolerate different concentrations of lead using three methods: agar dilution, gradient plate, and minimal inhibitory concentration (MIC).

Sampling Sites and Collection of Soil Samples

Cement dust-contaminated soils/sediment samples were collected from different locations around cement industrial zones in the Satna region, randomly selected at a depth of 0-25 cm, and stored in clean, labeled plastic bags. The digested soil samples were subjected to heavy metal analysis by ICP-MS, which showed lead and chromium with the highest concentration in the contaminated soil sample. In this paper, a focus on lead metal has been given.

2.2. PRIMARY SCREENING OF BACTERIAL ISOLATES FOR TOLERANCE TO LEAD-METAL

To isolate lead-tolerant bacterial strains, we used a conventional spread plate and streak plate procedure on agar media at 37°C for 24-48 hours. The colonies were selected based on visual observation and then streaked on Petri plates containing 15mg/kg of lead metal. The plates were incubated at 37°C for 48 hours. The same colonies were then picked up and streaked on sterile nutrient agar plates amended with 25mg/kg of lead. After incubation, the final tolerant colonies were obtained and further secondary screened for tolerance to lead metal using the MIC (Minimum Inhibitory Concentration) method (Yakoubi et.al, 2017).

2.3. EFFECT OF LEAD ON MICROBIAL GROWTH THROUGH VARIOUS METHODS

The tolerant bacterial isolates were further screened for their tolerance level to lead metal using several screening techniques, including the agar dilution method, gradient plate method, and MIC determination.

2.3.1. AGAR DILUTION METHOD

The lead tolerance capacity of the isolates was determined by gradually increasing the concentration of lead (2mg/L each time) from 15 mg/L onwards on metal-enriched nutrient agar plates until the strains failed to grow and the growth pattern was observed after incubation (Washington and Sutter).

2.3.2. GRADIENT PLATE METHOD

The effect of lead on the growth pattern of the bacterial strains was investigated using the gradient plate method. This method involved creating a gradient of lead concentrations on agar plates. The growth pattern was observed visually by measuring and comparing the colonial growth pattern on the plate.

2.3.3. MINIMUM INHIBITORY CONCENTRATION (MIC) DETERMINATION OF SOIL'S METAL-TOLERANT BACTERIA

The MIC determination involved preparing solutions of lead in vitro at increasing concentrations (2mg/L each time) from 15 mg/L onwards and incubating them with separate batches of test cultured bacterial isolates. The optical density (O.D.) of the lead metal-enriched broth tubes was measured at each step of increasing concentration at 680nm.

3. RESULT AND DISCUSSION

The results showed that the bacteria were able to tolerate high concentrations of lead and that the MIC method was the most effective in evaluating their tolerance. We also found that the bioremediation process was able to remove lead from contaminated soil, making it suitable for agricultural use. Overall, this study highlights the importance of bioremediation in removing heavy metals from contaminated soil and suggests that this approach could be a viable solution for addressing environmental pollution and public health concerns.

3.1. EFFECT OF LEAD ON MICROBE'S GROWTH BY VARIOUS METHODS

This study examines how lead exposure affects the growth of microbes using different experimental methods. Understanding these effects is crucial for assessing environmental risks and developing strategies to mitigate lead pollution's impact on microbial ecosystems.

3.1.1. AGAR DILUTION METHOD

The soil isolates exhibited a high level of tolerance to lead, with MTC values ranging from 15 to 33 mg/kg. In the BCN-B1 sample, isolates BCN-B1/5/Pb and BCN-B1/9/Pb were the most tolerant to lead, with an MTC of 33 mg/kg. In the PCN-P1 sample, isolate PCN-P1/1/Pb was the most tolerant to lead, with an MTC of 31 mg/kg.

3.1.2. GRADIENT PLATE METHOD

The soil isolates displayed a high level of sensitivity and tolerance to lead. The increasing sensitivity of colonies to the increasing concentration of metal is evident from the behavior of the developed pattern of test cultures. The plates showing no growth were recorded as the MIC metal gradient agar spread plate. Isolates BCN-B1/5/Pb and BCN-B1/9/Pb were the most tolerant to lead, with an MTC of 33 mg/kg. In the PCN-P1 sample, isolate PCN-P1/1/Pb was the most tolerant to lead, with an MTC of 31 mg/kg.

3.1.3. MINIMUM INHIBITORY CONCENTRATION (MIC) DETERMINATION OF SOIL'S LEAD-TOLERANT BACTERIA

The data revealed that almost all metal-tolerant bacterial strains tested showed considerable tolerance to lead, with MIC values ranging from 15 to 33 mg/L. Isolates BCN-B1/5/Pb and BCN-B1/9/Pb were the most resistant to lead, with a MIC of 33 mg/L. In the PCN-P1 sample, isolate PCN-P1/1/Pb was the most tolerant to lead, with a MIC of 31 mg/L.

A graph of Comparative MIC analysis of selected isolates for bioremediation is shown in Figure 1.

Figure 1

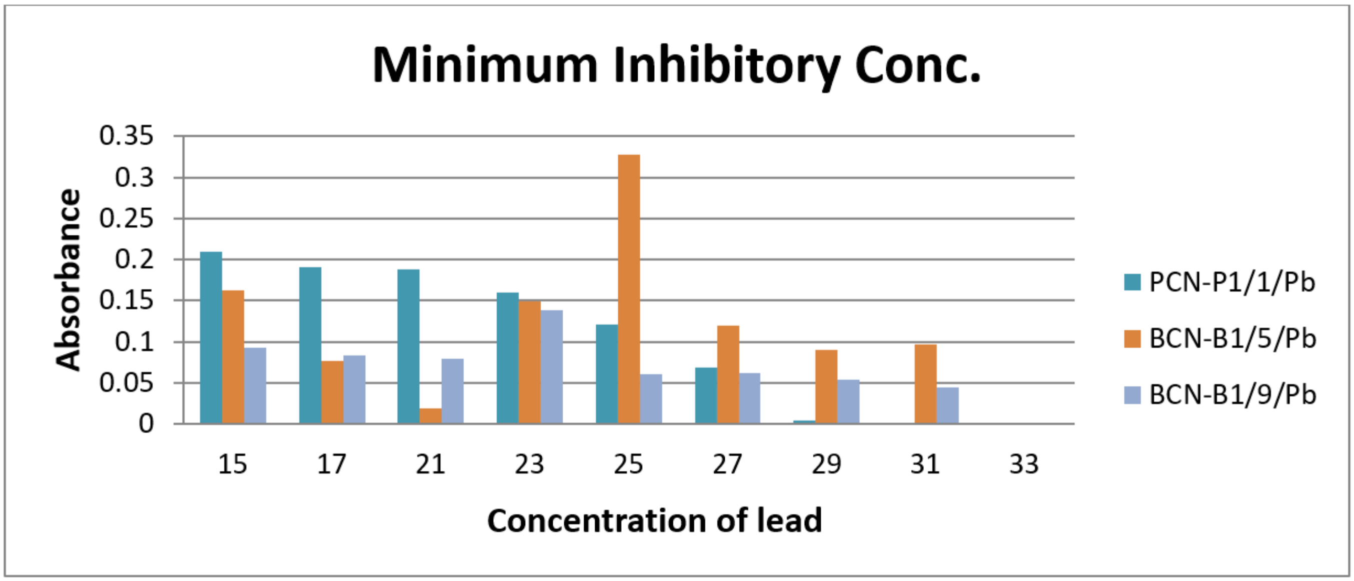

Figure 1 Comparative MIC Analysis of Selected Isolates for Bioremediation

4. DISCUSSION

A total of sixteen lead-tolerant strains were screened, selected, and studied from the soil of cement plants. On further secondary screening for their tolerance to Pb, it was observed that there was a decrease in the number of tolerant isolates at higher concentrations of Pb. The isolates taken for further studies were BCN-B1/5/Pb, BCN-B1/9/Pb, and PCN-P1/1/Pb which showed negligible or no colonial growth at 33 mg/l, 33 mg/l, and 31 mg/l concentration of lead, were the most tolerant with their MIC values respectively. This indicated inhibition of growth of the bacterial isolates at higher concentrations of heavy metals. Similar observations about the toxic effect of higher concentrations of heavy metals on the growth of bacteria have been reported.

The study found that high concentration of metals makes them more poisonous, have a smaller stimulating impact, and affect the system eventually severely impacted or impeded (Waoo et. al., 2014). This is likely due to synergistic effects in enzymatic catalysis. The sensitivity and tolerance of these bacteria to metals is probably related to incorporating those elements into the cytoplasm, once they bind to specific metallothionein.

The impact of heavy metals on the diversity of the bacterial community in the soil and its activities is related to their solubility and bioavailability which depends on a large number of soil parameters (Sharma and Raju, 2013). The presence of heavy metals in the soil leads to the disappearance of the most sensitive populations and subsequently to the adaptation of the most resistant/tolerant populations (Turpeinen et al., 2004). However, the considerable level of tolerance power to lead observed in the bacteria studied could be attributed to the important concentration of this metal in this soil (Aboushanab et. al., 2007).

It has been reported that the greater ability of these genera to grow in soil that contains numerous heavy metals, may be related to the fact that they contain different resistance functions such as intra and extra-cellular sequestration and efflux systems (Gupta et al., 2012; Bushra et al., 2016), or many predicted open reading frames involved in stress responses (Pal et al., 2014; Gómez-Ramirez et al., 2015).

5. CONCLUSION

The study isolated high lead-tolerant strains from the soil of cement plants, demonstrating the natural metal tolerance of bacteria in a contaminated environment. The MIC results indicated that the isolates were able to tolerate high concentrations of lead. These findings suggest that bacteria can develop mechanisms to resist the toxic effects of heavy metals, which could be exploited in bioremediation applications.

The study also highlighted the importance of monitoring environmental pollution and implementing remediation strategies to mitigate the negative impacts of heavy metal contamination. The contaminated soil of the Satna cement plant is an ideal ecological niche for tolerant lead bacteria and might be a source of bacteria with high bioremediation potential.

In terms of heavy metal contamination, it was observed that the cement industry could be a potential source of pollution. The excessive levels of metal concentration confirmed that this region has been stricken by activities of cement enterprises resulting in excessive metal contents in comparison to their background levels. It is recommended from the results of the current research that adequate monitoring of this source of pollution ought to be carried out regularly to stop the terrible effects of pollution. Increased industrialization has resulted in environmental contamination by lead in many soil systems. Therefore, lead-resistant isolates could be an interesting tool as an environmental marker. The results of this study demonstrate the potential for bioremediation as a sustainable and eco-friendly approach to cleaning up contaminated soil.

6. FUTURE ASPECTS

Satna city and nearby areas are one of the most important cement industrial hubs in the country. These areas comprise many enterprises and therefore, there may be a massive threat of environmental contamination which is exceptionally risky to our wellbeing. Many hazardous sites in the industrial areas in Satna require remediation. Yet, the truth of the matter is that industries and their businesses are expected for the economic system modernization and improvement and development of a metropolis like Satna.

Future research should focus on developing bioremediation techniques that can effectively remove heavy metals from polluted soil. This could involve studying the metabolic and absorption mechanisms used by bacteria to accumulate and transform heavy metals, as well as developing risk assessment plans for bioremediation applications. Additionally, further research is needed to explore the potential of using lead-resistant bacteria as environmental markers for monitoring pollution levels.

The study also highlights the need for continued monitoring and regulation of environmental pollution in areas affected by industrial activities. The use of bioremediation techniques could be an effective way to reduce the environmental impact of heavy metal pollution, particularly in areas where traditional remediation methods are not feasible.

Overall, this study demonstrates the future potential of bioremediation as a sustainable and eco-friendly approach to cleaning up contaminated soil and highlights the need for further research into this area.

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