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STUDY AND IMPORTANCE OF INDIGENOUS LEAD-RESISTANT BACTERIA FOR BIOREMEDIATION OF TOXIC HEAVY METAL SITES

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ABSTRACT

This study explores the significance of indigenous lead-resistant bacteria in the bioremediation of contaminated sites with toxic heavy metals. Heavy metal pollution poses serious environmental and health risks, compelling effective remediation approaches. Indigenous bacteria, adapted to their local environments, exhibit natural resistance mechanisms that enhance their potential for detoxifying lead and other metals. This research involves isolating lead-resistant bacterial strains from contaminated sites, characterizing their resistance traits, and assessing their bioremediation capabilities through various techniques. From the biochemical test conducted, two bacterial isolate viz; Bacillus carotarum and Bacillus lentus were isolated from four soil samples collected in Sa'adatu Rimi College of education Agriculture departmental farm. From the pH result obtained of two soil samples, A and B at concentration of 10^{-6} and 10^{-7} shows soil pH 7.91, 7.89 respectively.

Keywords: Bioremediation, Heavy metal, Indigenous bacteria, Lead-Resistant Bacteria

INTRODUCTION

Environmental pollution has been recognized as one distillery, fertilizer units, electroplating plants, tannery of the major problems of the modern world (Liu et al., 2017). The increasing industries, sago factories, oil refineries, pesticide and demand for water and dwindling supply has made the herbicide industries (Kulshreshtha et al., 2010). These industrial effluents containing treatment and reuse of industrial effluents attractive heavy metals pose a serious threat to the ecosystem. The problem of environmental pollution on Use of these industrial effluents and sewage sludge for account of essential industrial growth is practical terms, agriculture have become a common practice in India as a the problem of disposal of industrial water, whether solid, result of which these toxic metals get transferred and liquid or gaseous. All the three types of wastes have the accumulated into plant tissues from soil (K et al., 2016). Metals are natural constituents of earth and some of them viz. Zn, Ni, Cu are essential for living organisms, but prove toxic to micro and macro organisms at higher concentrations (Mohan & Dubey, 2013). However, Lead, cadmium and mercury are toxic to bacterial cells even at low concentrations (Pachaiyappan et al., 2021). The lead toxicity can serve as the perseveres in environment that cannot be degraded or destroyed (Fashola et al., 2016). Lead is a toxic heavy ions that has a stark harmful influence to humanoid health and environment in general that damage the central nervous system of the brain and resulted to serious low IQ depression in children and death, mostly in children do to the high level of absorption compare to adults (Lanphear, 2005).

The data recorded by WHO (Thirulogachandar et al., 2014), it has been estimated that heavy lead metal ion harming resulted in one hundred and forty three thousand (143,000) deaths, and Contribute to six hundred thousand 2 (600,000) fresh cases of children with intellectual inabilities each year. However, a research by Chatterjee et al., (2014), point out the level of toxic heavy metals contamination in water as a general delinquent around the globe due to anthropogenic activity by industries and lead ore processing company (Thirulogachandar et al., 2014). Nevertheless, it is clearly evident that the preceding methods of bioremediation are flexible and known to be successful in reinstalling contaminated sites with different pollutant types where microbes play a crucial role in the remediation process. Consequently, the variety and abundance of community systems in contaminated environments provide insight into the fate of any bioremediation technique, providing that additional environmental factors can hinder bacterial activity and maintain the optimum range (Chibueze *et al.,* 2016). Ideally, these techniques require less expensive compared to techniques used in ex-situ bioremediation as no extra costs needed for quarry processes; however, the rate of designing and installing some sophisticated equipment on site to enhance microbial activities during bioremediation is of major concern (Thwaites et al., 2020). Chemical substances discharged into the environment may be natural or can be anthropogenic due to the rapid industrialization and widespread use of compounds containing metals that are non-biodegradable and have a long residence time in the environment cause serious eco-toxicological problems and are infamous for their tendency to bioaccumulation as depicted in table fig. 1 (Chatterjee et al., 2014).

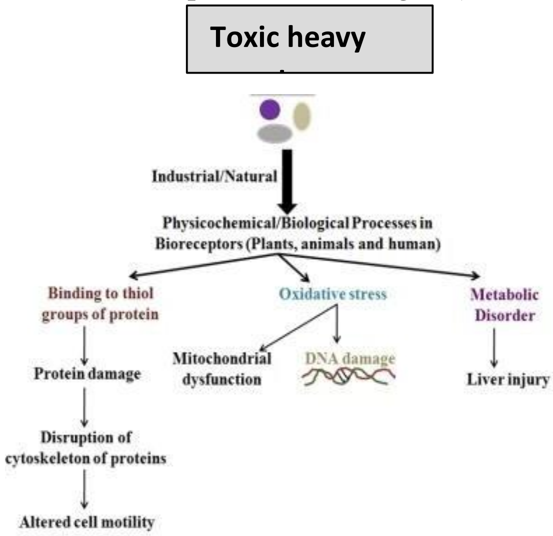


Figure 1: Biological processes in plants, animals, and humans in response to heavy metal exposure.

These research will focus on the new modern technology by using microorganisms in solubilizing the toxic heavy metals into less toxic form thereby immobilizing them using bacteria that could find option for detoxifying the heavy metals in the environment through the use of some bacteria species that are resistant bacterial strains capable of modifying the contaminant that could be utilized and purify the waste soil effluents into non-toxic form (Lovley et al 200).

MATERIALS AND METHODS

Media. The medium used for the isolation and growth of lead-resistant bacteria was Nutrient agar.

Site selection

The soil samples were collected aseptically in sterile plastic bags, by taking the upper surface of 1-2 inches with well-defined depth procedure from Sa'adatu Rimi College of education Agricultural farm, Kano state Nigeria, between June and July, 2024, and preserved at 4°C for further Analysis.

Serial dilution technique

A 1g of soil sample was suspended in 9ml sterile distilled water and mixed vigorously with 1ml micropipette for 1 to 2 minutes making the total volume 10ml test tube. The ten test tubes were labeled 10^{-1} to 10^{-10} respectively. 1ml of the sample labeled 10^{-1} were transfer into 10^{-2} -10^{-10} respectively by making the total volume of each test tube 10ml as per standard method of MacLowry et al., (1970), with minor modifications. The Serially diluted soil samples were inoculated on solidified Nutrient agar and incubated for 24 to 72 h at 37^{0}C.

Screening of heavy metals resistant Bacteria

The bacterial isolates were primarily identified by Gram's staining technique and later confirmed by biochemical assays, and the purity of the strains was verified by microscopic examination

Identification of heavy metals resistant Bacteria

Lead resistant bacteria isolate were identified and studied using different cell morphology, physiological and biochemical characteristics tests of the bacterial cells, as per standard protocol of Bergey's Manual of determinative bacteriology Oyetibo *et al.*, (2010).

Resistance to heavy metal ions

Stock solutions containing 10 mg/mL of lead nitrate, cadmium chloride, and other metal salts copper sulfate, zinc sulfate, potassium dichromate, and nickel chloride, were used to assess the cross-heavy metal resistance of the bacterial isolates. A cross metal resistance test was performed. Increasing the amount of the relevant metal present in progressively, checking for metal concentrations of 50 µg/mL. Metal ions were added to culture flasks holding 100 mL of media, Overnight 20 µL bacterial colonies, and then incubated at 37 degrees celsius for 24 h. The optical density at which growth was observed was 600 nm using spectrophotometer.

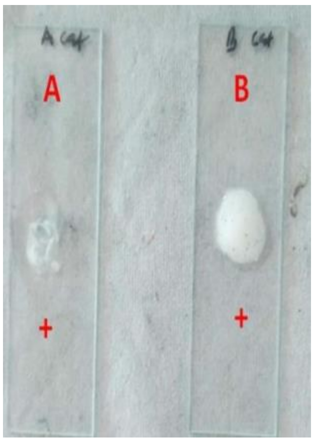
Morphological Studies

Bergey's systematic bacteriology manual shows that each isolate's colony morphology and culture characteristics have been systematically studied. A total of twenty strains were isolated; among them, two were recovered from the soil.

Catalase Test

The test were carried out to observe the presence or absence of enzyme activity in the study. However, bubble formations were observed, which indicate the presence of positive test, after smearing a loopful bacterial culture on the surface of the slide, with immediate addition of 2-3 drops of hydrogen peroxide (H202).

Figure 2: Showing bubble formation of catalase test for sample **A** and **B** **Starch hydrolysis test**



Bacteria culture were grown on petri plates containing starch agar, with single Zigzag streaking and was incubated at $37^{0}C$ for 24 to 48 h of sample A and B. Iodine solution were flooded to the surface of solidified media on petri plate with a dropper after incubation, and was allowed to hydrolyze for 30 seconds, a clear zone around the bacterial growth was shown, and that confirmed the presence of Bacillus subtilis.

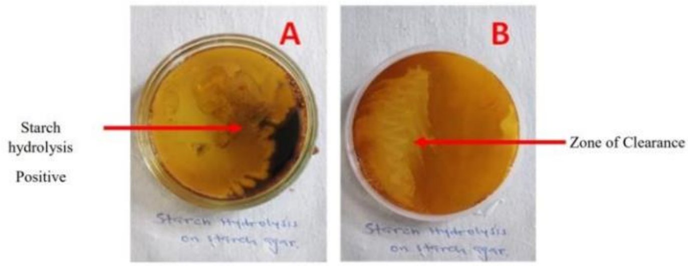


Figure 3: Starch hydrolysis test of sample A and B on starch agar showing zone of clearance

Antibiotics sensitivity test

The paper disc method was adopted for determining the antibiotics sensitivity patterns of isolates. Standard antibiotic disks of septrin 20 µg (SXT), chloramphenicol 200 µg (CH), sparfloxacin 10 µg (SP), ciprofloxacin 5 µg (CPX), amoxacillin 10 µg (AM), augmentin 20 µg (AU), gentamycin 10 µg (CN), pefloxacin 20 µg (PEF), Tarivid 5 µg (OFX), streptomycin 20 µg (S), ampiclox 10 µg (APX), zinnacef 10 µg (Z), rocephin 15 µg (R), erythromycin 5 µg (E), metronidazole 20 µg (MTZ), cefuroxime 20 µg (CXM), amoxycillin 20 µg (AMC), and ceftriaxone 20 µg (CRO) were placed on Mueller–Hinton agar already seeded with 0.1 ml inoculum of isolates. Plates were incubated at 37^0C for 24 to 72 h for zones of inhibition to be observed.

RESULTS

This research work focused on the new modern bioremediation technology using microorganisms that will solubilize the toxic heavy metals resistant bacterial strain into less toxic form. Metals exert their toxic effects on microorganisms through various mechanisms. Even at micro molar concentrations, cadmium and lead inhibit the growth of most wild-type bacteria and are tolerated by only a minority of microorganisms. Nevertheless, we have isolated 20 resistant bacteria from polluted soil which are tolerant. These 20 resistant bacteria, two were found to be resistant, the finding is in consistent with (Vassilev et al., 2004). The primary screening of bacterial isolation was performed using serial dilution technique of two soil samples A and B, NA media were used in the isolation. The spreaded pour petri plate were incubated at 37^0C for 24 hours as reported by Umrania *et al.,* (2006). Primary screening of the two soil sample A, and B were achieved using serial dilution technique as described by (Desai, 2017). The isolated colony found in concentration of 10^{-4} to 10^{-6} petri plates were selected and was streak at the surface of new prepared NA media in an aseptic condition, and incubated at 37^0C for 24 h as reported by (Loeschcke et al., 2013). The catalase test was carried out to observe the presence or absence of enzyme activity by adding 2-3

drops of hydrogen peroxide (H202), and a bubble formations were observed, that the bacteria cells produce Catalase positive, which is in consistent with the finding of Bertrand et al, (2002); Gummersheimer & Giblin, (2014). Also, for starch hydrolysis test, bacteria culture were grown on petri plates containing starch agar with single, Zig-Zag streaking and was incubated for 24 to 48 h at 37^0C for both sample A and B. Iodine solution were flooded to the surface of petri plate, and was allowed to hydrolyze for 30 seconds, iodine solution as shown in figure 3, transparent clear zones around the colonies was shown, as iodine forms the colored complex with starch that indicates a clear zone around the bacterial growth and confirmed that Bacillus subtilis is positive in the lead soil samples, The result is in line with findings of (Sree et al., 2019).

Statistical analysis

Observations were made, and all the experiments run in triplicate. At least three separate flasks were usually maintained for one treatment. The average of control and experimental groups were compared and significant differences evaluated by using Student’s t test of significance.

CONCLUSION

In the present investigation, it was concluded that out of 20 Bacterial isolates only two isolates viz. *Bacillus cereus and Bacillus carotarum* show a significant range of resistivity of heavy metals. That shows, indigenous lead-resistant bacteria represent a promising avenue for addressing heavy metal contamination through bioremediation. Their natural adaptations and mechanisms significantly mitigate environmental pollution, offering sustainable solutions for contaminated sites.

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Conflicts of Interest: There is no competing interest in relation to this manuscript

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