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Elucidate the Influence of Heavy Metal on Bacterial Growth Isolated from a Mining Location and A Waste Dump: Using their Inducible Mechanism

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Abstract

Heavy metals from industry and solid waste dumping site activity are accumulating in soils at alarming levels because of global industrialization. Microorganisms have evolved a number of methods to cope with such high levels of heavy metals. The goal of the study was to use heavy metals' inducible mechanism to elucidate the effect of heavy metals on bacterial growth isolated from a mining site and a waste dump site in liquid broth. A total of 91 rhizobacteria were isolated, with 51 from the Zawar mines site in Udaipur, Rajasthan, and 40 from the Pirana garbage site. Ahmedabad is a city in Gujarat, India. Six rhizobacteria (SMHMZ2, SMHMZ4, SMHMZ46, SMHMP4, SMHMP23, and SMHMP38) showed increased resistance to heavy metals. Temperature 37°C and pH 7 were found to be optimal growth conditions for all six isolates. The growth curves of each culture were initiated using 1% inoculum WM & W/OM in 200ml nutrient medium containing the appropriate metal concentration Ni^{2+} (1500 ppm), Pb^{2+} (1000 ppm), and Cd^{2+} (500 ppm) against which we had to verify the tolerance of each culture. At various time intervals after the start of the development curve for each culture, OD values at 600 nm spectrophotometrically (Shimadzu, Model No.1722) were acquired. The lag period is minimized in all bacterial isolates, including SMHMZ2, SMHMZ4, SMHMZ46, SMHMP4, SMHMP23, and SMHMP38, since inoculums created with metal after four subsequent transfers became adapted due to the establishment of an inducible mechanism towards heavy metal tolerance. Isolated heavy metals that tolerate rhizobacteria could be useful as bioremediation agents and in groups working on heavy metal phytoremediation in contaminated environments.

Keywords: Heavy metals, Nickle, Cadmium, Lead, Mining area, Landfill site, Growth curve, bioremediation etc.

Introduction

The accumulation of hazardous metals from ore tailings dumping and leachate overflow in and around mining areas is currently creating concerns about environmental stress. Mining activities, particularly open-pit mining, often produce the most solid wastes in the form of waste rock. Acidic mine drainage (AMD) discharged with high amounts of heavy metals can contaminate downstream water, agricultural soils, food crops, and biota, posing a health concern to populations living near mines [1-3]. Mining-related heavy metal contamination is a serious global environmental hazard, particularly in developing countries [4]. According to a Times of India storey about a municipal solid waste dumpsite, the Pirana Landfill site takes approximately 4000 tonnes of rubbish per day, with the majority of it being deposited in the landfill untreated. Furthermore, hazardous chemical waste and by-products generated by a number of industries/factories in the vicinity of the landfill site were dumped in a haphazard manner [5]. Because of their high toxicity, cadmium and lead are considered among the main contaminants [6,9]. Mine tailings, effluents from the textile, leather, tannery, electroplating, and galvanising industries, as well as cadmium batteries, all discharge cadmium into the environment [10]. Furthermore, cadmium is a reasonably common element that is not present in its pure form in nature. Steel plating, stabilizers for polyvinyl chloride colours in plastics and glassware, electrode material in nickel–cadmium batteries, and as a component of various alloys and ceramics are some of its main applications [11]. At low concentrations, heavy metals such as Ni, Co, and Pb, however, cause oxidative stress, lipid peroxidation, carcinogenesis, mutagenesis, and neurotoxicity in people, animals, and plants [12]. The discharge of heavy metal-containing effluents puts pressure on the ecosystem, posing health risks to plants, animals, aquatic life, and humans. Toxic metals are transferred to groundwater and bioaccumulated after surface contamination [13,14]. When certain amounts of heavy metals are introduced into the environment, the bulk of the micro flora dies, leaving only a few cells with metal resistance mechanisms. Heavy metals can cause significant changes in microbial populations and their activity when they are introduced into the environment in various forms [15]. Several research have looked into the heavy metal sensitivity or resistance of bacteria isolated from various habitats, as well as their processes for adapting to the hazardous metal during exposure [16,17]. Some microbes have been reported to have evolved heavy metal detoxification systems, and some even exploit them for respiration, making them resistant to them [18]. Permeability barriers, intracellular and extracellular sequestration, efflux pumps, enzymatic detoxification, and reduction are some of the inducible mechanisms used by microbes to cope with heavy metal stress [19]. The use of heavy metal-tolerant rhizobacteria to boost metal bioavailability in heavy metal-polluted soil is a promising strategy. The goal of this work was to extract and characterize rhizobacteria that tolerate Cadmium, Lead, and Nickel from heavy metal-polluted soil. Isolated heavy metal tolerant rhizobacteria could be useful as bioremediation agents and in conjunction with phytoremediation of Cd^{2+}, Pb^{2+}, and Ni^{2+} contaminates in soil.

Material and Methods

Bacterial culture

A total of 51 rhizosphere bacteria were recovered from the Zawar mines in Udaipur, Rajastha [20], while 40 rhizosphere bacteria were obtained from the Pirana trash site in Ahmedabad, Gujarat. The bacterial colonies SMHMZ2, SMHMZ4, and SMHMZ46 from the Zawar mines, as well as SMHMP4, SMHMP23, and SMHMP38 from the Pirana landfill site, showed better resistance to heavy metals (Ni^{2+}, Pb^{2+}, and Cd^{2+}).

Chemicals and media used

The media employed in this investigation came from Hi Media Laboratories in Mumbai, India, and includes nutrient agar compositions such Peptone, Beef extract, Yeast extract, and Agar powder. NaCl, $(NH_4)_2SO_4$, K_2HPO_4, KH_2PO_4, $CaCl_2$, $FeSO_4$, $MgSO_4$, and glucose were furnished by Fine Chemicals (P) Ltd. in New Delhi, India. Heavy metal standards such as $NiCl_2.7H_2O$, Pb $(NO_3)_2$, and $CdCl_2$ were given by SRL Pvt. Ltd., Mumbai, India.

Heavy metal stock solution preparation

We tested the tolerance of 91 bacterial isolates obtained from rhizosphere soil from mines and landfill sites to three heavy metals Ni^{2+}, Pb^{2+}, and Cd^{2+}at various concentrations. Ni^{2+} (5000µg/ml), Pb^{2+} (5000µg/ml), and Cd^{2+} (5000µg/ml) heavy metal stock solutions were prepared using $NiCl_2.7H_2O$, Pb $(NO_3)_2$, and $CdCl_2$, respectively. All of the above-mentioned heavy metal solutions were sterilised by autoclaving for 15 minutes at 15 psi pressure and 121^{0}C [20]. The growth rates of all six isolates were assessed using growth cubes in the presence and absence of respective heavy metals. To begin each culture's growth curve, two types of inoculums were used.

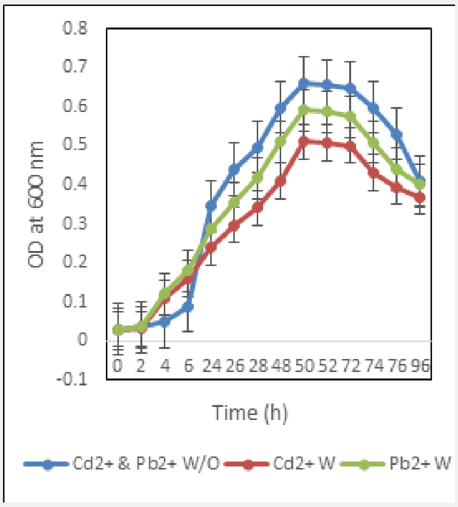

Figure 1: Growth curve of Culture SMHMZ2.

Development of the inoculum

Inoculum with metal (WM) was created with 3 mL Nutrient medium and 1% culture, as well as the above-mentioned metal concentration, at a temperature of 37^{0}C and 150rpm on a spinning shaker, with pH 7. As a result, Ni^{2+}, Pb^{2+}, and Cd^{2+} inoculums have been developed for each culture. To increase each culture's adaptability to heavy metals (in this case, Ni^{2+}, Pb^{2+}, and Cd^{2+}) in Nutrient medium, we repeated the technique and circumstances for inoculum development with metals four times for all cultures. All six cultures' inoculum WM in Ni^{2+}, Pb^{2+}, and Cd^{2+} entered log phase within 2-6 hours in the subsequent four transfers. In subsequent transfers, the time it takes for each culture to reach the log phase has decreased. 3 mL Nutrient medium and 1%

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culture were used to make inoculums without metal (W/OM). In this situation, metal-free inoculums have been established for all cultures. In the absence of heavy metals, all cultures reached their exponential phase within 2-12 hours.

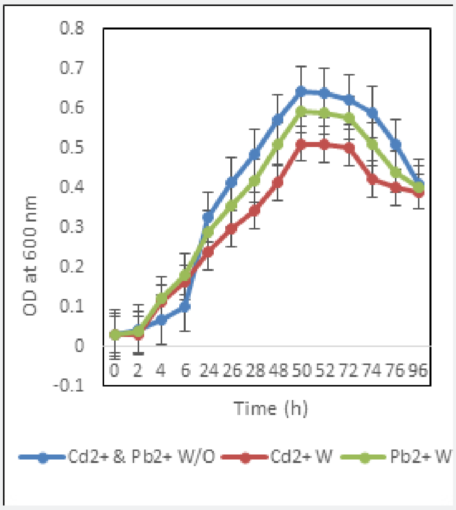

Figure 2: Growth curve of Culture SMHMZ4.

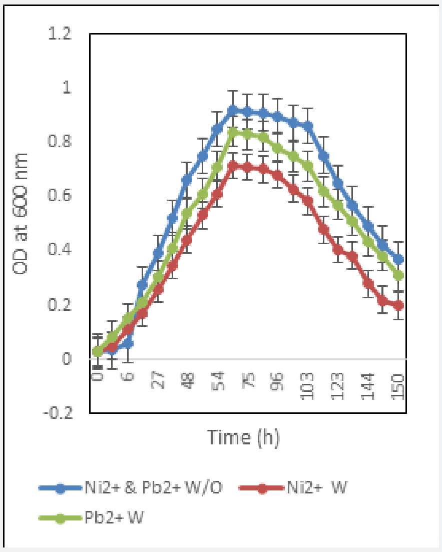

Figure 3: Growth curve of Culture SMHMZ46.

Growth Studies

To assess the ideal growth conditions in terms of pH and temperature, all bacteria isolates were grown in nutrient medium with a pH of 7, at a temperature of 37^{0}C and 150rpm on a spinning shaker. Following the development of both types of inoculums, the growth curves of each culture were started using 1% inoculum WM & W/OM in 200ml nutrient medium containing the desired metal concentrations Ni^{2+} (1500 ppm), Pb^{2+} (1000 ppm), and Cd^{2+} (500 ppm), against which we had to check the tolerance of each culture. At various time intervals after the start of the development curve for each culture, OD values at 600 nm spectrophotometrically (Shimadzu, Model No.1722) were acquired. As a result, for each of the six cultures, we have two growth curves for each metal, one starting with inoculum generated WM and the other starting with inoculum W/OM.

Result and Discussion

In the presence of all three metals Ni^{2+}, Pb^{2+}, and Cd^{2+} the lag phase of bacterial cultures SMHMZ2, SMHMZ4, SMHMZ46, SMHMP4, SMHMP23, and SMHMP38 is reduced when the growth curve is started with inoculum developed in the presence of metal, compared to growth curves started with inoculum developed

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without metal, where the lag phase is increased. As a result, the findings show that inducible proteins play a role in the tolerance mechanisms displayed by bacterial isolates SMHMZ2, SMHMZ4, SMHMZ46, SMHMP4, SMHMP23, and SMHMP38. Inoculums formed in the lack of metal induced the extended lag phase in the development curve. As a result, the metal acts as an inducer, causing bacteria to make inducible proteins that help them evolve and tolerate their environment. The lag period is minimized in the cases of bacterial isolates SMHMZ2, SMHMZ4, SMHMZ46, SMHMP4, SMHMP23, and SMHMP38 because inoculums created with metal after four subsequent transfers became adapted due to the establishment of an inducible mechanism towards heavy metal tolerance. After inoculation into new Nutrient media, their development commences rather quickly (with a shorter lag period) due to a previously described inducible mechanism.

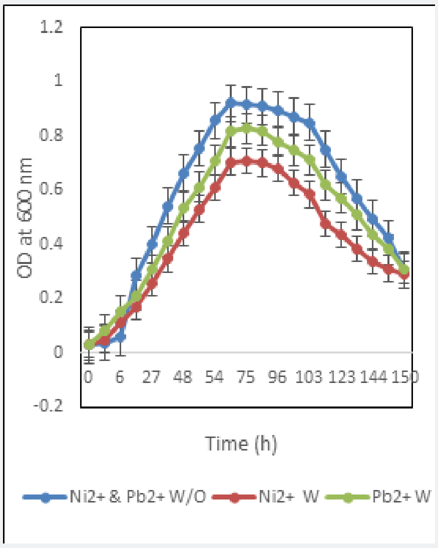

Figure 4: Growth curve of Culture SMHMP38.

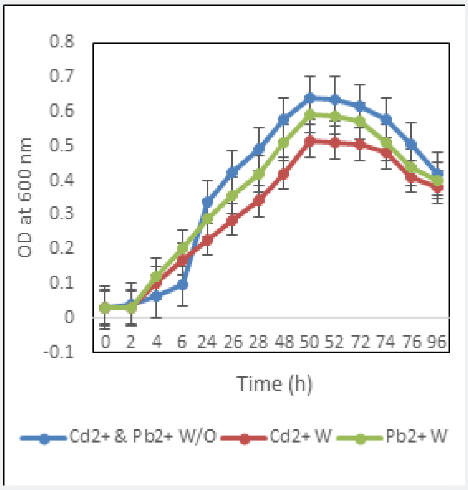

Figure 5: Growth curve of Culture SMHMP4.

Conclusion

In polluted soil samples from a mining location and a waste dump site, we discovered 91 rhizosphere bacterial species, according to our research. Six bacterial strains with potential in heavy metal resistance and bioremediation were identified after testing on greater concentrations of cadmium, lead, and nickel. The growth kinetics showed that bacterial cultures SMHMZ2, SMHMZ4, SMHMZ46, SMHMP4, SMHMP23, and SMHMP38 were more tolerant. As a result, the metal acts as an inducer in the culture,

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resulting in the synthesis of inducible proteins and a protein inducible mechanism in the SMHMZ2, SMHMZ4, SMHMZ46, SMHMP4, SMHMP23, & SMHMP38, lowering the lag phase even in the presence of significant heavy metal concentration.

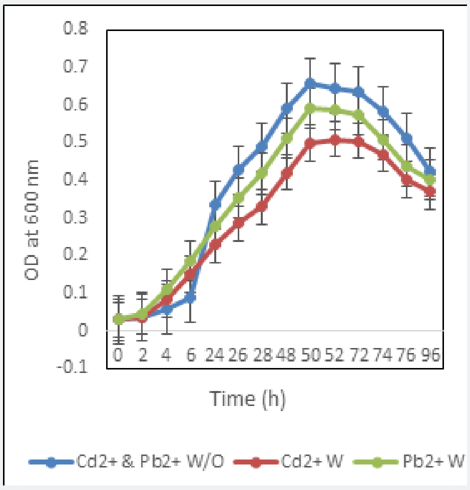

Figure 6: Growth curve of Culture SMHMP23.

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