Volume : 4 | Issue : 11 | November 2015 • ISSN No 2277 - 8179

Research Paper

Isolation and Characterization of Lead Metal Resistant Bacteria for Its Prospects in Bioremediation of Contaminated Soil

Microbiology

KEYWORDS : Bioremediation, Lead resistant, Bacillus spp.

Sapale G. G	Department of Microbiology Kankavli college, Kankavli Dist: Sindhudurg Maharashtra
Patil.S.B	Department of Microbiology Kankavli college, Kankavli Dist: Sindhudurg Maharashtra
Raorane C.J	Department of Microbiology Kankavli college, Kankavli Dist: Sindhudurg Maharashtra
Sawant N	Department of Microbiology Kankavli college, Kankavli Dist: Sindhudurg Maharashtra

ABSTRACT

Introduction of Heavy metals like Lead, Chromium, Cadmium, Nickel, Copper, and Cobalt into the Natural habitat like soil, water and air causes significant environmental problem. Lead (Pb) is a major pollutant found in soil water and air is hazardous and highly toxic to human, animal, plants and Microorganisms. Interestingly some microorganisms like Staphylococcus spp. Escherichia. Coli, Flavobacterium spp., Bacillus spp are having ability to detoxify such heavy metals so play role with cleaning up or remediating heavy metal contaminated environment. Present study deals with isolation identification and characterization of the heavy metal resistant bacteria were isolated from sewage water followed with the study of lead tolerance and bioremediation. In initial screening five bacterial isolates were selected out of this two bacterial isolates shows high Lead resistance as well multiple antibiotic resistance ability might be possible to use in bioremediation.

INTRODUCTION:

Heavy metal pollution of soil and wastewater leads significant environmental problem. Wastewater arises from the industries and sewage sludge applications have permanent toxic effect to human and environment. Lead is a major pollutant that is found in the soil water and air is hazardous waste which is highly toxic to Animals, plants, and microorganisms. Bioremediation remediates the environment like contaminated soils and water with bacteria. Bacteria which are carried out the bioremediation are mostly resistant to the contaminant; such resistant was developed by various mechanisms, and correlated with the Antibiotic resistance. Bacterial species had been isolated from the drinking water that was tolerate to metals and antibiotics simultaneously (*Rinoy, Varghese et. Al.2012)*. Such resistance was mostly generated due to the acquired ability to tolerate the metals by various mechanism like Efflux of the metals and antibiotics, Intracellular bioaccumulation, Precipitation, Extracellular sequestering, Biosorption (*Sabyasachi Chatterjee et al 2013)* Heavy metals like the Lead Arsenic and Cadmium Contaminated to the Soil water and air which are hazardous to the life leads to the Mental retardation in children which are also inhibits the growth of microorganisms. Interestingly some bacteria have the ability to detoxify such metals (*Khan et. Al 2009)*. Lead occurs in four different valence states Pb^0, Pb^+, Pb^{2+}, and Pb^{4+} within them Pb^{2+} is more soluble and bioavialable under condition of low P^H, Low organic content, low levels of particulate matters and low concentration of the salts of calcium, Iron, Manganese, zinc and Cadmium (Harrison et.al 2009). Some bacteria like *staphylococcus spp*., *Klebsiella spp, E.coli* show Chromium resistance, *Acenitibacter*, *Flavobacterium spp*. And *Citrobcter spp* shows cadmium resistance and its possible to use for bioremediation purpose In most of the such cases heavy metal resistance is associate with the antibiotic resistant because gene encoding antibiotic resistance and metal resistance present on the same plasmid. (*Amalesh Samanta et at.2012).*

MATERIALS AND METHODS:

Collection of environmental samples:

Sampling sites:

Soil samples as well as surface water sample were collected from several solid waste dumping sites from various points of waste discharge. And different sites of Ulhas River, located near the Ulhasnagar Samples were collected in sterile polycarbonate bottles and used within 24 hrs of collection for physicochemical and bacteriological analysis.

Also sample was collected from Common Effluent treatment plant located at Koparkhairane Vashi; Thane-Belapur region is collected and treated. The bottles containing water samples of effluent were mechanically shaken prior to use and kept for 10 minutes to allow the heavy particles to settle down. Physicochemical analysis of water and soil samples was done to determine pH (using digital pH meter), temperature (using mercury thermometer) as per the standard procedures. The appropriate volume of water sample was taken for physicochemical and bacteriological analysis

Enrichment of the collected samples:

Each 10 g soil sample was added to 90 ml sterilized water and mixed on the magnetic blender for 30 min to separate bacteria from the soil completely. After being deposited for 20 min, 1 ml suspension was added to NB broth and incubated at 30°C for 24hrs on 120 rpm. Heavy metals incorporated into media were used. The NB incorporated with lead heavy metal was prepared. The concentration of each heavy metal was maintained at 300 µg/ml of the medium

Isolation of lead Resistant Bacteria:

Bacterial strains were isolated from soil and effluent of Metal Sewage. Isolation was achieved by serial dilution method. Followed with a loop full sample was streaked to sterile Nutrient agar medium plates and spread properly in three different petriplates or in one by making segments, as available. The plates were then kept for incubation at 37°C for 24 – 48 hrs. The concentration of each heavy metal was maintained at 300 µg/ml of the medium

Determination of threshold level of metal resistance

For the determination of MIC, heavy metal resistant bacterial isolates were grown in NB tubes with gradually increasing the concentration of the respective heavy metal. The initial minimum concentration used was 300µg/ml. Each Subsequent transfer to NB agar plates with increasing the metal concentration until the isolate failed to grow. This concentration above which bacterial isolates failed to grow was considered as threshold level of metal resistance.

Identification and Characterization of Bacterial Isolates:

From sample five morphologically different isolated colonies

were selected after 48 hrs of incubation. All the isolates were placed for identification, characterization as per the Bergey's manual of determinative Bacteriology (Holt *et.al.* 1994).

Determination of environmental optimum conditions (pH, temperature, and salinity and carbon sources) for growth of lead resistant bacteria

Carbon source:
Carbon sources viz. glucose were tested in nutrient medium in order to select best carbon source required for lead resistance. Overnight grown culture was inoculated in fresh growth media (pH7) to a starting optical density of 0.02 at 600 nm. Bacterial isolates were grown in the presence of different carbon sources (0.4 %) at 30°C , 150 rpm and growth was monitored after 24 hrs by measuring absorbance at 600 nm.

P[H:]

Bacterial growth at various pH values from 5 to 9 were tested to investigate optimum pH required by the lead resistant bacterial isolate for growth and lead resistance in nutrient broth media amended with lead acetate. Bacterial isolates were grown at different pH values at 30°C and 150 rpm. Here 0.4% glucose was used as carbon source and growth was monitor after 24 hrs by measuring absorbance at 600 nm (0.D)

Temperatures:
Bacterial growth at various temperatures ranging from 0-40°C were tested to investigate best temperature required by the lead resistant bacterial isolates for growth and lead resistance in nutrient medium with lead acetate. Here 0.4% glucose was used as carbon source and growth was monitored after 24 hrs with constant shaking at 150 rpm by measuring absorbance at 600 nm (O.D).

Salinity:
Bacterial growth at various NaCI concentration ranging from 0.5-3% were tested to investigate best NaCI concentration required by the lead resistant bacteria for growth and lead resistance. Bacterial isolates were grown at different medium with lead acetate. NaCl concentration at pH 7.0 and 30°C with constant shaking at150 rpm. Here 0.3% glucose was used as carbon source and growth was monitored after 24 hrs by measuring absorbance at 600 nm.

Association of metal resistance with multiple drug resistance:
Antibiotic susceptibility test of lead resistant bacterial isolates was performed following Kirby—Bauer disc diffusion method (Bauer et al., 1966) using Muller—Hinton agar and antibiotic discs (Himedia, India).Various antibiotics tested are as follows: chloramphenicol(C), gentamycin(G), ,ampicillin (A), Cloxacillin (AX), neomycin (N), nitrofurantoin (Nf) , nalidixic acid (Na), ciprofloxacin (Cf), cephaloridine (Cr), Oflaxacin (OF) and Diameter of zone of inhibition of test isolates caused by individual antibiotics was compared with standard chart (Himedia) and based on these results resistance or sensitivity of bacterial isolates to the tested antibiotics was determined.

Effect of isolates on different metals:
The isolates found were tested for the resistance using different metal ions. Medium used was nutrient agar containing different metal salts. Different metals used were zinc chloride, copper sulfate, mercuric chloride, manganese chloride. 24 hrs old cultures of Isolates were spread against antibiotic disc and Incubated at 37° C for 24 hrs

Immobilization of lead resistant bacteria:
Freely-suspended microbial biomass has disadvantages that include small particle size, low mechanical strength and difficult biomass/effluent separation. Immobilized biomass particles in packed- or fluidized-bed reactors minimize these disadvantages (Macaskie & Dean, 1989; Gadd & White, 1990). Immobilized living biomass has mainly taken the form of biofilms on supports made of a range of inert materials. Lead resistant bacteria were immobilized in form of calcium alginate beads of uniform size and allowed to harden at 4^0 C.

RESULT AND DISCUSSION:
Isolation of lead Resistant Bacteria:
Bacterial strains were isolated from lead contaminated soil. Different morphological isolates were found. In which five colonies were screened from initial level of heavy metal supplemented NA medium. Out of different bacterial culture, five bacterial isolates were selected and purified. As per the Bergey's manual of determinative bacteriology, the selected isolates were studied for the morphological, cultural and biochemical test the isolate G1 is found to be *Bacillus spp* & G2 *is Proteus spp* respectively which are used for further study.

Optimization of growth parameters: P[H]

The pH was optimized to for maximum growth in presence of respective metal at a

threshold concentration of the selected bacterial isolates. Growth response of bacterial isolates at different pH was analyzed by spectrophotometericaly at 600 nm where optimum pH for isolate G1was 7.0 while optimum pH for isolate G5 was 7.0

Temperature
Selected bacterial isolates found to show different characteristic growth on nutrient broth at different temperature. So temperature was optimized for maximum growth of the bacterial isolates. G1 was found optimum at 37^0C temperature whereas G2 also was found to be optimum at 37^0 C.

Salt concentration
Selected bacterial isolates found to show different characteristic growth on nutrient broth at different salt concentrations. So salt was optimized for maximum growth of the bacterial isolates in the presence of respective metal at threshold concentration indicates that and 0.5% was optimum for both the isolate

Glucose concentration
Selected bacterial isolates found to show different characteristic growth on nutrient broth at different glucose concentrations. So salt was optimized for maximum growth of the bacterial isolates in the presence of respective metal at threshold concentration indicates that and 0.5% was optimum for both the isolates

Association of metal resistance with multiple drug resistance:
After incubation the plates for 24 hrs at 37°C zone of clearance was observed around the antibiotic disc. The zone of clearance was measured and compared it with the standard Kirby bauers chart of antibiotics the result observed as follows:

Table 1: Antibiotic sensitivity table

Sr. No	Antibiotics used	*Bacillus Spp*	*Proteus Spp*
1	Aztreonam (AO)	resistant	intermediate
2	Ampicillin(AMP)	intermediate	intermediate
3	Nitrofurantoin (NIT)	resistant	resistant
4	Trimethoprim (TR)	sensitive	resistant
5	Ciprofloxacin(CIP)	sensitive	sensitive
6	Gentamycin (GEN)	intermediate	intermediate
7	Neomycin (N)	resistant	resistant
8	Chloramphenicol (C)	intermediate	sensitive
9	Ofloxacin (OF)	sensitive	sensitive
10	Cloxacillin(AX)	sensitive	resistant

Effect of different metal on isolates:

The isolates found were tested for the resistance with different metal ions. Medium used was nutrient agar containing different metal salts. Hence the *Bacillus spp* and Proteus *spp* were found resistant to both mercury and manganese metal.

Table12: Effect of different metal on isolates

Metal salt used	*Bacillus spp*	*Proteus spp*
$ZnCl_2$	sensitive	sensitive
$CuSO_4$	sensitive	sensitive
$HgCl_2$	resistant	resistant
$MnSO_4$	resistant	resistant

Immobilization of lead resistant bacteria:

Freely-suspended microbial biomass has disadvantages that include small particle size, low mechanical strength and difficult biomass/effluent separation. Hence the isolates obtained are immobilized forming calcium alginate beads. Immobilized biomass particles in packed- or fluidized-bed reactors minimize these disadvantages. Variety of bioreactor configurations including rotating biological contactors, fixed bed reactors, trickle filters, fluidized beds and air-lift bioreactors used which can further used for bioremediation.

CONCLUSION:

Bacillus strains resists 1200mg/L lead acetate and alteration in cell morphology as reduction in cell size when exposed to lead acetate. And *Proteus spp* strain found resist 1600mg/L lead acetate. Optimization with different growth parameters was done. Temperature, pH, glucose concentration and salt concentration are different parameters used. Immobilized Heavy metal resistant microorganisms play an important role in the bioremediation of heavy metal contaminated soils.

REFERENCE

[1] Amalesh Samanta, Paramita Bera, Mahamuda Khatun, Chandrima Sinha, Pinaki Pal, Asif Lalee, Anurup Mandal , An investigation on heavy metal tolerance and antibiotic resistance properties of bacterial strain Bacillus sp. isolated from municipal waste, J. Microbiol. Biotech. Res., 2012, 2 (1):178-189 [2] Bauer AW, Kirby WMM, Sherris JC, Turck M (1966). Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. 45 (4): 493-496 [3] C. Edward raja , g. s. selvam and kiyoshi omin, isolation, identification and characterization of heavy metal resistant bacteria from sewage., journal of biotechnology vol. 12(32),pp. 5069-5074, 7 august, 2013 [4] C.J.Raorane, C.C.Gavimath,G.P.Kukreja, D.P.Kalsekar, S.M.Kulkarni, B.E.Ravishankar, R.S. Holi, Isolation, identification & phylogenetic analysis of phthalic acid degrading bacteria., International journal of advanced biotechnology and research.,2012,3 (4) : 804-809 [5] Gadd GM (1990). In Microbial Mineral Recovery (Ehrlich, HL and Brierley, CL., Eds.), McGraw-Hill, New York, pp.249-275. [6] Holt JG, Krieg RN, Sneath AHP, Staley TJ, Williams TS (1994). Bergey's Manual of Determinative Bacteriology, 9th Edition. (International Edition) [7] Hossein Zolgharnein, Mohd Lila Mohd Azmi, Mohd Zamri Saad, Abdul Rahim Mutalib, Che Abd Rahim Mohamed, Detection of plasmids in heavy metal resistance bacteria isolated from the Persian Gulf and enclosed industrial areas, Iranian journal of biotechnology, vol. 5, no. 4, october 2010. [8] Meghraj hookoom and Daneshwar puchooa,Isolation and Identification of Heavy Metals Tolerant Bacteria from Industrial and Agricultural Areas in Mauritius,Current Research in Microbiology and Biotechnology Vol. 1, No. 3 (2013): 119-123 [9] Rinoy Varghese, Krishna M. P., Arun Babu V. and A. A. Mohamed Hatha Varghese et al.,Biological removal of lead by bacillus sp. Obtained from metal contaminated industrial area, Current Research in Microbiology and Biotechnology 2012 : 2 (2) 756-770 [10] Sabyasachi Chatterjee, Anindita Mukherjee, Agniswar Sarkar, Pranab Roy., Bioremediation of lead by lead-resistant microorganisms, isolated from industrial sample Advances in Bioscience and Biotechnology, 2012, 3, 290-295