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Determination of Minimum Inhibiotry Concentration and Antibiotic Suspecibilty of Zinc Resistant Bactreia from Industrial Waste Water and Soil

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ABSTRACT

Environmental pollution with toxic heavy metals is spreading throughout the world along with industrial process and accumulated in soil and waste water. Microorganism have developed several mechanism to tolerate such high concentration of heavy metal for this purpose screening of bacteria having tolerance to heavy metal zinc had been attempted in the present study. Total six zinc resistant bacteria isolated from the industrial waste water and soil. Based on morphological and biochemical characterization isolates were identified as Staphylococcus aureus. (W1, S1) Bacillus Spp. (W2, S2), and E.coli (W3, S3). Minimum inhibitory concentration (MIC) of the isolates were studied and it was found that E.coli (W3) strain have high resistance against zinc (MIC-1200µg/ml). The antibiogram was done and it was found that metal resistant bacteria exhibited high resistant pattern towards a group of antibiotics.

Keywords: Zinc resistant, Minimum inhibitory concentration (MIC) ,Antibiogram.

I. INTRODUCTION

The most abundant pollutants in industrial waste water and soil are heavy metals, these are the elements with molecular weight and their atomic number greater than 20. Screening and characterization of heavy metal resistant bacteria for its prospect in bioremediation of contaminated soil (Joshi, B. H., & Modi, K. G. 2013). Some heavy metals are toxic to living organisms at a high concentration. Some metals like zinc, Copper, Manganese, etc. they are essential for the normal healthy growth and reproduction of living organism but at a high concentration this is trace elements damages cell membrane, DNA, enzyme activity of the living organisms.

Toxic heavy metal like Hg, Cu, Cr, Pb, Cd, Zn have no biological role they are well known for the toxicity mutagenic and carcinogenic impact on human being and other living system (El-Deeb, B. 2009). The main source of heavy metals is industrial activities such as metal processing, mining and electroplating, tanning, carpet washing and drying .The introduction of heavy metals in various forms in environment can result in considerable modification of microbial communities and their activities (Sheik, C. S. et al., 2012). Microbes are the capability of consuming organic waste. When the microorganisms consume these waste, they converts waste into the nontoxic material. In this process of conversation they actually produce many metabolites to degrade the complex waste into the simple compound. This is because microorganism have developed resistance mechanism to survive in presence of toxic heavy metals. (Mustapha MU and Halimoon N 2015). The bioremediation techniques are effective and efficient for the remediation of pollutants so as the bioremediation technology from laboratory to field to clean up the environment can be taken up. For bioremediation microorganism must be enzymatically attached pollutants and convert them to harmless products (Pandey, B. Fulekar, M.H. boil 2012).

The resistance mechanisms used by microorganism to tolerate heavy metal stress include permeability barriers,

intra and extracellular sequestration efflux pumps enzymatic detoxification and reduction. In some cases resistance to metal ions has been reported to be plasmid mediated and observed to be encoded by genes in close proximity to antibiotic resistance gene (Nikaido H., 2009). The ideal solution for pollution abatement is "bioremediation " which is the most efficient strategy to manage and recover the contaminated environment (Ahemad and Khan 2011).The term bioremediation has been introduced to exploit the biological traits of organisms for removing toxic species from the polluted site (Vidali, 2001). The aim of this study was isolation and identification of zinc resistant bacteria, determination of the resistance spectrum by measuring the minimum inhibitory concentration and to study antibiogram of the isolated bacteria.

II. MATERIALS AND METHOD

Collection of sample

The experiment was conducted using industrial waste water and soil sample collected from MIDC area, Jalna (MS). Water samples were collected in a sterile Screw caps bottle and soil sample collected into zip lock bags, brought into the laboratory and were stored at 4°C for further study.

Isolation and identification of zinc resistant bacteria

The zinc resistant bacteria was isolated from industrial waste water and soil a sample by inoculating the metal 50 mg/liter concentration in nutrient agar medium. The isolation was achieved by serial dilution method. 1ml waste water and 1 gm soil sample was added in which 9 ml of sterile saline. The dilution was prepared upto 10^{-6}, then 0.1 ml of the higher dilation was spread on the surface of agar plates and incubated at 37°C for 24 hours. Zinc resistant bacterial colonies were selected and identified by standard identification methods (Holt et al., 1994).

Minimum inhibitory concentration (MIC)

All the isolates were checked for metal tolerance by growing on nutrient agar (NA) medium containing zinc. The initial concentration used was 50µg/ml and thereby gradual increasing the concentration each time on NA plate's from 50µg/ml to 1200µg/ ml. the plates were incubated for 24 hours. The lowest concentration that prevented bacterial growth was considered the MIC.

Antibiogram of the bacterial isolates

All the isolates were tested for antibiotic sensitivity according to Kirby-Bauer disc diffusion method (Bauer et al., 1996). Antibiotic disc containing penicillin (10units), Azithromycin (15mcg), Vancomycin (30mcg), Cefazolin (30mcg), Clindamycin (2mcg), Erythromycin (15mcg), tecoplanin (30mcg) was used. Plates of Muller Hinton agar (Himedia) medium having media up to 4 mm were prepared. After solidification lawn of inoculum was prepared on to agar plates for each isolate. Inoculum was taken by socking the sterile swab (Himedia) in prepared inoculum of the isolate and spread over the agar plates for respective organism. The antibiotics disc were placed on plates and incubated at 37 ^{0}C for 24 hours. (Nath et.al. 2012) .After incubation, the organisms were classified as sensitive or resistant to an antibiotic according to the diameter of inhibition zone given in standard antibiotic disc chart.

III. RESULT AND DISCUSSION

Isolation and identification of zinc resistant bacteria

The six different isolates were observed on nutrient agar incorporated with zinc metals which were resistant to zinc. They were identified as E.coli (S3, W3), Bacillus spp., (S2, W2) and Staphylococcus aureus. (S1, W1).

Minimum inhibitory concentration

The six isolates were further referred for MIC counts, the bacteria showing minimum inhibitory concentration for heavy metals ranging from 50µg/ml- 1200µg/ml. The detailed information are given in table 1.

Table 1. Minimum inhibitory concentration (S = soil sample, W= water sample)

Bacterial Isolate	Strain Name	Source	MIC
Staphylococcus aureus.	S1	Soil	300µg/ml
	W1	Water	550µg/ml
Bacillus spp.	S2	Soil	600µg/ml
	W2	Water	500µg/ml
E.coli	S3	Water	900µg/ml
	W3	Water	1200 µg/ml

The minimum inhibitory concentration was shown highest by E.coli (W3) strain i.e.1200µg/ml and the lowest was found in Staphylococcus aureus. (S1) i.e. 300µg/ml).

Antibiogram of zinc resistance bacteria

The bacterial isolates were tested for the antibiotic sensitivity. The predominant isolates that are tolerant to zinc were found to be multi drug resistant (S1, S2, S3, W1, W2, and W3). Some strains were resistant and some were sensitive to selected antibiotic (table No. 2). Some of the isolates were resists to Azithromycin, Clindamycin, Cefazolin, Erythromycin, Penicillin antibiotics and some are sensitive to Teicoplalin, Vancomycin and Cloxacillin. The isolates in our study were not only resistant against zinc but also resist to multiple antibiotics. It is well reported that microorganism have affinity for metals and can accumulate them in by various mechanism (Ahemad M. & Malik A. 2011).

Table 2. Antibiogram of zinc resistance bacteria

Bacterial Isolate	Strain Name	Sensitive	Resistant
Staphylococcus aureus	S1	Vancomycin, Teicoplalin,	Cefazolin, Penicillin, Erythromycin, Teicoplalin,
	W1	Vancomycin, Teicoplalin, Erythromycin, Cefazolin. Clindamycin,	Azithromycin, Penicillin, Cloxacillin.
Bacillus spp	S2	Vancomycin, Teicoplalin, Erythromycin	Azithromycin, Clindamycin, Cefazolin, Penicillin, Cloxacillin,
	W2	Teicoplalin, Vancomycin. Azithromycin, Clindamycin, Cefazolin, Erythromycin, Cloxacillin,	Penicillin
E.coli	S3	Teicoplalin ,Vancomycin, Cloxacillin,, Penicillin,	Azithromycin, Clindamycin, Cefazolin
	W3	Teicoplalin ,Vancomycin, Cloxacillin,, Penicillin,	Azithromycin, Clindamycin, Cefazolin

As bioremediation can be effective only where environmental condition permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at faster rate. These factors include existence of microbial population capable of degrading the pollutants, the availability of contaminants to microbial population the environmental factors (Singh, S.*et al.*, 2015). Heavy metal exerts their toxic effect on microorganism through various mechanism and metal tolerant bacteria could survive in these habits and possibly isolated and selected for the potential

application in the bioremediation of contaminated sites. Thus, the application of microbial populations specifically adapted to high concentrations of heavy metals will increase the ability to remediate heavy metal contaminated soils. The results of present study showed that the metal resistant bacteria can be used for heavy metal remediation.

IV. REFERENCES

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