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Isolation, characterization and antibiogram of copper resistant bacterial strain from industrial waste water and soil.

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Abstract

The pollution of the environment with heavy toxic metals is spreading all through the world along with industrial progress. Microorganism have developed several mechanisms to tolerate high concentration of heavy metals, for this purpose screening of copper resistant bacteria has been attempted in these study. Total five copper resistant bacteria were isolated from industrial waste water and soil sample. Based on morphological and biochemical characterization these isolates were identified as *Bacillus spp*., (S1, W1*), Pseudomonas spp*.,(S2) and *Staphylococcus aureus*. (W2, W3).The MIC of the isolates were tested with varying concentration of copper from 50 µg/ml to 1200µg/ml. The antibiogram was done, it was found copper resistant bacteria exhibited high resitant towards group of antibiotics.

Keywords: Copper resistant, minimum inhibitory concentration, Antibiogram.

Introduction

Heavy metals consistent a very heterogeneous group of elements widely varied in their chemical properties and biological function. Heavy metals are kept under environmental pollutant category due to their toxic effects on plants, animals, Human Being. Heavy metal are present in nature and gets accumulated in soil and plant. Dietary intake of many heavy metals through consumption of plants has long term detrimental effect on human health.Some heavy metal have bio-important as trace element but the biotoxic effect of many of them in human biochemistry are of great

concern, hence there is a need for proper understanding of mechanism involved,such as the concentration and oxidation states, which makes them harmful.It is also important to know there sources, leaching processes and chemical conversion and their modes of deposition in polluting the environmental,which essentially supports life,poisoning and toxicity in ecosystem occurs frequently through exchange and co-ordination mechanism.(Mahurpawar., 2015). As trace elements, some heavy metals (e.g. copper, selenium, zinc) are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning. Heavy metal poisoning could result, for instance, from drinking-water contamination (e.g. lead pipes), high ambient air concentrations near emission sources, or intake via the food chain.Environmental pollution is global threat of increasing severity due to urban growth, industrialization and changing life styles (Lago et al., 2015). Many researches have been conducted on heavy metals contamination in soil from various sources such as industrial waste, automobile emission and agriculture practice. Excessive levels of heavy metals likes CO, Cu, Pb, Ni and Hg are considered as mainly toxic pollutants (Gurave et al., 2015). Toxic metals, to a large extent, are dispersed in the environment through industrial effluents, organic wastes; refuse burning, and transport and power generation. They can be carried to places many miles away from the sources by wind, depending upon whether they are in gaseous form or as particulates. Metallic pollutants are ultimately washed out of the air into land or the surface of water ways. Thus air is also a route for the pollution of environment. Metal containing industrial effluents constitute a major source of metallic pollution of hydrosphere. In humans, the Cu is essentially needed but in high doses it causes anemia, liver and kidney damage, and stomach and intestinal irritation may occur. During Wilson's disease, it affects greatly. It is normally found in drinking water from Cu pipes and additives designed to control the algae growth. (Pandey et al., 2014)

Bacteria are among the most abundant organism that occurs everywhere on earth. Heavy metals are increasingly found in microbial habitats due to several natural and anthropogenic processes; therefore, microbes have evolved mechanisms to tolerate the presence of heavy metals by either efflux, complexion, or reduction of metal ions or to use them as terminal electron acceptors in anaerobic respiration. The microorganisms respond to these heavy metals by several processes; including transport across the cell membrane, bio sorption to the cell walls and entrapment in

extracellular capsules precipitation, complexion and oxidation-reduction reactions (Nath, et al., 2012). The microbes essentially breakdown complex, often toxic substance into simple beings from which they can utilize in there metabolic processes for growth (Mihdhir et al., 2016).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 (Bhaig El-Deeb 2009). The Resistance mechanisms used by microorganism to tolerate heavy metal stress include permeability barriers, intra and extracellular sequestration efflux pumps enzymatic detoxification and reduction (Nikaido, H. 2009).The term bioremediation has been introduced to exploit the biological traits of organisms for removing toxic species from the polluted site (Vidali, 2001). The objective of present study was to isolate and identify thecopper resistant bacteria from waste water and soil.

Materials and method

Collection of sample

The experiment was conducted using the industrial waste water and soil samples collected from the MIDC area, Jalna.Water samples were collected in sterile bottle and soil samples were collected in zip bags,brought to the labarotory and stored at 4°Cfor further study.

Isolation and identification of Copper resistant bacteria.

Copper resistant bacteria was isolated from a sample by inoculating the metal 50 µg/ liter concentration of solution by serial dilution method the waste water and soil sample in which 9 ml of sterile saline in 6 test tubes than 1 ml of sample was added to the first test tube to have 10^{-1} repeated up to 10^{-6} then 0.1 ml of the higher dilution was spread on the surface of agar plates and incubated at 37° C for 24 hours. Bacterial strains were isolated from soil and effluent of Metal Industries by chemical and morphological characterization was done by standard identification method (Holt et al., 1994).

Minimum inhibitory concentration (MIC)

All the 5 isolates were checked for metal tolerance. The initial concentration used was 50µg/ml and thereby gradual increasing the concentration each time on nutrient agar plate's from 50µg/ml to 1200µg/ ml. the plates were incubated for 24 hours. The gradual increase the concentration of heavy metal each time on nutrient agar plates until strains failed to give colonies on plate. The lowest concentration that prevented bacterial growth was considered the MIC.

Antibiogram of the bacterial isolates

Isolated copper resistant isolates were tested for antibiotic sensitivity and resistance according to Kirby -Bauer disc diffusion method (Bauer et al., 1966). Antibiotic disc containing penicillin (10units), azithromycin (15mcg), vancomycin (30mcg), cefazolin (30mcg), Clindamycin (2mcg), Erythromycin (15mcg), tecoplanin (30mcg) was used. The culture was spread on nutrient agar plates. The antibiotics disc were placed on plates and incubated at 37^0C for 24 hours. (Nath et al., 2012). After incubation, the organisms were classified as sensitive or resistant to antibioticaccording to the diameter of inhibition zone given in standard antibiotic disc chart.

Result and discussion

Isolation of Copper resistant bacteria

The five different isolates were observed on nutrient agar incorporated with Copper metal which was resistant to copper. After isolation of copper resistant bacteria identification was done on the basis of morphological and biochemical characteristics. They were identified *Pseudomonas spp, Bacillus spp.* and *Staphylococcus spp.*

Minimum inhibitory concentration

The five isolates were further referred for minimum inhibitory concentration. The concentration of copper metals ranging from 50µg/ml- 1200µg/ml was used to determine the MIC.The detailed information are given in table no.1.

Table No.1: MIC of the isolates.

Bacterial isolate	Strain name	Source	MIC
Staphylococcus aureus	W2	Water	1200 µg/ml
	W3	Water	850 µg/ml
B. Subtilis	S1	Soil	750 µg/ml
	W1	Water	900 µg/ml
Pseudomonas Spp.	S2	Soil	1050 µg/ml

Antibiogram of Copper resistance bacteria

All bacterial isolates were tested for the antibiotic sensitivity. The predominant isolates that are tolerant to copper were found to be multi drug resistant (S1, S2, W1, W2, W3). The some strains were resistant and some were sensitive to some antibiotic. all the isolates were resistant to Teicoplanin antibiotic.

Table no 2: Antibiotic sensitivity testing

Bacterial Isolate	**Strain Name**	**Sensitive**	**Resistant**
Staphylococcus aureus	W2	Cloxacillin,Vancomycin,Clindamycin,Cefazolin, Penicillin.	Teicoplanin,Azithromycin, Erythromycin.
	W3	Cloxacillin,Vancomycin,Clindamycin,Cefazolin,	Teicoplanin,Azithromycin, Erythromycin,Penicillin
Bacillus spp	S1	Penicillin, Vancomycin , Coloxacillin, Azithromycin . Clindamycin,Cefazolin,	Teicoplanin, , Erythromycin
	W1	Vancomycin ,Coloxacillin, . Clindamycin,Cefazolin	Teicoplanin, , Erythromycin. Azithromycin, Penicillin
Pseudomonas Spp.	S2	Vancomycin ,Coloxacillin, . Clindamycin,Cefazolin,	Teicoplanin,Azithromycin, Erythromycin.

		Penicillin	

The five isolates were isolated from the bacterial strains which were identified by morphological characterization. The bacterial isolates from soil irrigated with metal polluted waste water were selected owing to their maximum selective growth and MIC. Copper resistant bacteria isolated from soil and waste water.. It is noticeably evident that domestic and industrial wastes toxic effects are the reason why bacteria developed resistance mechanisms along with the hazard of human health and environment. (Mustapha et al., 2015). Most of the isolates were resistant to Teicoplalin, Erythromycin, Azithromycin. It is well report that microorganism have affinity for metals and can accumulate them in by various mechanism (Ahemad et al., 2011).

it is well reported that microorganism have affinity for metals and can accumulate them in by various mechanism (Andreazza, et al., 2011). Copper is one of the toxic heavy metals of Concern in the environment. Toxicity of heavy metals is largely due to their presence in aqueous system in ionic forms which are easily absorbed by living organisms. There is increasing interest in the use of microbial biomass for visitation of heavy metals form the environment. Biosorption of heavy metals involve accumulation of the metal's in microbial biomass with subsequent recovery and remediation through bioremediation or chemical technologies. Copper resistant microorganism with capacity to absorb copper on biomass can be used as bioremediation tools to remove copper from contaminated terrestrial and aquatic environment.

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