RESEARCH ARTICLE

Isolation and Characterization of Cadmium Resistant Bacteria From Industrial waste water and Soil

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Heavy metal contamination in the environment has become a serious problem due to the increase in the addition of metals to the environment. Heavy metals such as cadmium are not readily absorbed or captured by microorganisms and become threat to an aquatic and environmental system. In Present study bacterial strains were isolated from soil and water samples taken from the metal contaminated industrial area and Cadmium resistance of the isolates were investigated. Seven cadmium resistant bacteria were isolated and identified as *Staphylococcus spp.(S1,W1)*,*Bacillus spp.(S3,W3)*,*Pseudomonas spp.(S2,W2)*, two remains unidentified on the basis of morphological and biochemical characteristics.Minimum inhibitory concentration (MIC) and antibiotic resistance pattern of the isolates was also studied. *Bacillus spp.*)W3) and *Staphylococcus spp.* (S1) were found to have high resistance pattern against Cadmium (550 µg/ml). It was observed that the metal resistance bacteria exhibited high resistance pattern towards a group of antibiotics.

Key words: Cadmium resistance, Minimum inhibitory concentration (MIC), antibiotics.

INTRODUCTION

Toxic heavy metal like Hg, Cr, Cu, Zn, Pb, Cd are well known for their toxicities, mutagenic and carcinogenic impact on human beings and other living system especially those metals classified under priority list of pollutants (Mustapha and Halimoon 2015). Natural resources including plants and microorganism are extensively explored to combat metal ion pollution (El-Deeb, 2009). Cadmium which are major contaminants found into the environments and extremely poisonous to humans, animals, plants and microbes which can damage cell membrane alter particularity of enzyme and destroy the structure of DNA (Marzan *et al.*, 2017). Cd and almost all of its compound are water soluble and hence easily gain entry in human food chain, no physiological role of cadmium in human cellular metabolism has been reported so far and it is extremely toxic in very minute quantity. It has also been reported to cause osteoporosis and fractures, anemia, eosinophilia, anosmia, apoptosis, diabetes mellitus,

oncogenes, activation and chronic pulmonary problem (Khan *et al.,* 2016). Cd is highly toxic non-essential as these are not needed for the survival of organism and are toxic in low concentrations. Cd is non-biodegradable heavy metal with half-life of 20 years. (Demirezen, 2006). The threat of heavy metals pollution to human and wild life has led to increased interest in developing system that can remove or neutralize heavy toxic metals in soil and waste water (Valls *et al.,* 2002). The cadmium cycle between river, water and sediments, the vegetation in the area and the metal levels in the blood and urine of residents in several studies have proven the direct and indirect impact of environmental pollution on human health. (Kafilzadeh et al., 2013). Cadmium particularly accumulates in renal, lung, pancreas, and liver and damages those (Nishijo *et al.,* 2006). The presence of high levels of heavy metals in the environment has an inhibitory effect on most microorganisms. However, microorganisms have evolved their resistance mechanisms that lead to the selection of resistant variables that can tolerate metal toxicity (Nasrazadani *et al.,* 201l). various conventional ways are used for removal of cadmium from waste water like electrochemical treatment, chemical precipi tation, ion exchange, reverse osmosis, membrane technology, phytoremediation, oxidation and reduc-tion are very expensive and not environmen-tally acceptable (Abbas *et al.,* 2014). Cadmium is one of the most toxic pollutants of the surface soil layer released into the environment by mining and smelting activities atmospheric deposition from metallurgical incinera-tion of plastics and batteries (Abbas et al. (2014). No treatment for cadmium toxicity has been approved so far. Several chemicals and physical methods are used to remove cadmium from industrial effluent prior to release the effluent into environment but all these methods are expensive and less effective. Bacteria remove heavy metal ions including Cd^{2+} from the environment either by metabolism independent absorption on their cell walls or metabolism depen-dent intracellular accumulation. Hyper accumulation of Cd^{2+} has been reported to disturb the cell physio-logy by reactive oxygen species (ROS) production and disruption of bacterial respiratory proteins.

The aim of this study was isolation and identification of cadmium resistant bacteria, determination of the resistance spectrum by measuring the minimum inhibitory concentration and to study antibiogram of the isolated bacteria.

MATERIALS AND METHODS

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°C for further study.

Isolation and identification of cadmium resistant bacteria
The cadmium metal was isolated from samples by inoculating the metal and the metal sample using concentration of $50\mu g$/liter. Isolation was achieved by serial dilution method. The waste water and soilsample was serially diluted in which 9ml of sterile saline in 6 test tubes then 1ml of sample was added to the 1st test tube to have 10^{-1} repeated up to 10^{-6} then 0.1ml of the higher dilution was spreaded on the surface of the agar plates and incubated at 37°C for 24hrs. Bacterial strains were isolated from soil and effluent of metal industries. Biochemical and morphological characters of the predominant bacterial genera were studied and finally characterized and identified by standard identification methods (Holt *et al.,* 1994).

Minimum inhibitory concentration (MIC)
All the Seven isolates were checked for metal tolerance. The initial concentration used was $50\mu g$/ml and thereby gradual increasing the concentration of heavy metal each time on nutrient agar (NA) plates until the strains failed to give colonies on the plate. The growth of cultures on last concentration was transferred to the higher concentration by streaking on the plate. The lowest concentration that prevented bacterial growth was considered the MIC.

Antibiogram of the bacterial isolates
Isolated heavy metal resistant isolates were tested for antibiotic sensitivity and resistance according to Kirby -Bauer disc diffusion method (Bauer *et al.,* 1996). Anti-biotic disc containing penicillin (10units), azithromy-cin (15mcg), vancomycin (30mcg), cefazolin (30mcg), Clindamycin (2mcg), Erythromycin (15mcg), tecopla-nin (30mcg) was used. The culture was spread on nutrient agar plates. The antibiotics disc were placed on plates and incubated at 37 °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.

MIDC area of Jalna. They were identified as *Pseudomonas spp., Bacillus spp.,* and *Staphylococcus spp.*

RESULTS AND DISCUSSION

The seven isolates of cadmium resistant bacteria were isolated from the industrial waste water and soil from

Minimum inhibitory concentration

The seven isolates were further referred for MIC count the bacteria showing minimum inhibitory concentration for heavy metals ranging from 50μg/ml- 600μg/ml. The detailed information is given in table 1.

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

Bacterial Isolate	Strain Name	Source	MIC
Staphylococcus spp.	S1	Soil	550μg/ml
	W1	Water	350μg/ml
Bacillus spp.	S3	Soil	450μg/ml
	W3	Water	550μg/ml
Pseudomonas spp.	S2	Soil	350μg/ml
Unidentified	W2	Water	500μg/ml
	W4	Water	500 μg/ml

The minimum inhibitory concentration was shown highest in W3 strain (550μg/ml) and the lowest were found in W1 and S2 (350μg/ml)

Table 2: Antibiogram of cadmium resistant bacteria

Bacterial Isolate	Strain Name	Sensitive	Resistant
Staphylococcus spp.	*S1*	Azithromycin,Vancomycin, Clindamycin, Cloxacillin.	Cefazolin, Penicillin, Erythromycin, Teicoplalin.
	W1	Teicoplalin, Cloxacillin.	Azithromycin, Vancomycin, Clindamycin, Cefazolin, Erythromycin, Penicillin.
Bacillus spp.	S3	Nil	Azithromycin, Vancomycin, Clindamycin, Cefazolin, Erythromycin, Penicillin, Teicoplalin, Cloxacillin.
	W3	Teicoplalin, Vancomycin.	Azithromycin, Clindamycin, Cefazolin, Erythromycin, Penicillin, Cloxacillin,
seudomonas spp.	S2	Teicoplalin, Vamcomycin, Erythromycin, Cloxacilln	Azithromycin, Clindamycin, Cefazolin, Penicillin.
Unidentified	W2	Vancomycin	Azithromycin, Clindamycin, Cefazolin, Erythromycin, Penicillin, Teicoplalin, Cloxacillin.
	W4	Vancomycin	Azithromycin, Clindamycin, Cefazolin, Erythromycin, Penicillin, Teicoplalin, Cloxacillin.

Antibiogram of cadmium resistance bacteria

The bacterial isolates were tested for the antibiotic sensitivity. The predominant isolates that are tolerant to cadmium were found to be multi drug resistant (S1, S2, S3, W1, W2, W3, and W4). Some strains were resistant and some were sensitive to some antibiotic results are given in table 2.

The antibiotics such as Azithromycin, Clindamycin, Cefazolin, Erythromycin, and Penicillin were resistant to the isolated strains cadmium resistant bacteria whereas the antibiotics Teicoplalin, Vancomycin, Cloxacillin were sensitive to some of the isolated strain. Multiple tolerance occurs only to toxic compounds that have similar mechanisms underlying their toxicity. Since heavy metals are all similar in their toxic mechanism, multiple tolerances are common phenomena among heavy metal resistant bacteria.

The result of this study indicates that bacteria isolated were *Staphylococcus spp., Bacillus spp., Pseudomonas spp.* As these species shows high tolerance to cadmium and are good candidates for the treatment and elimination of cadmium polluted rivers. Heavy metal resistant microorganisms play an important role in the bioremediation of heavy metal contaminated soils (Ray and Ray, 2009). Cd is considered as one of the most toxic heavy metals and they can appear either in water or soil of any polluted site because of their high mobility, especially in agricultural fields, thus greatly threatens human health via food chains (Goris *et al.,* 2001). Heavy metals exert their toxic effects on microorganisms through various mechanisms, and metal-tolerant bacteria could survive in these habitats and possibly be isolated and selected for their potential application in the bioremediation of contaminated sites (Piotrowska-Seget *et al.*, 2005).

The concentration of a toxic metal that affects the growth and survival of different microorganisms varied greatly. It is clearly indicated that domestic waste and industrial waste are responsible for the development of bacterial resistance along with the risk of human health and environment. These bacteria helps to formulate bioremediation agent to detoxifying tannery effluent at industrial waste water and soil. Metals are of special concern for the environment and agro-ecosystems, for they appear to be particularly dangerous because of their high toxicity patterns, their extremely long residence time in ecosystems and their ubiquitous distribution worldwide, as pointed out

before. As many researchers have mentioned, common physical and chemical methods used to remove heavy metals from waste water samples are expensive and time consuming (Nasrazadani *et al.*, 2011). 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, from the present study it can be concluded that the application of microbial populations specifically adapted to high concentrations of heavy metals will increase the ability to remediate heavy metal contaminated soils.

Conflicts of interest: The authors stated that no conflicts of interest.

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