JJBS
Jordan Journal of Biological Sciences

Volume 10, Number 2,June 2017
ISSN 1995-6673
Pages 101 - 107

Putative Mechanism of Cadmium Bioremediation Employed by Resistant Bacteria

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Received: December 3, 2016 Revised: April 9, 2017 Accepted:April 16, 2017

Abstract

Cadmium is one of the non-essential and toxic heavy metals which affect the terrestrial and aquatic biota along with human beings due to its release from industrial effluents directly into terrestrial and aquatic ecosystem. The bioremediation of heavy metals using microorganisms has emerged as a substitute for the physicochemical techniques in recent years. So, the present study deals with the isolation and screening of heavy metal resistant bacteria from three different locations of battery manufacturing sites of Faridabad industrial area, Haryana, India. In this study, five bacterial isolates were selected based on high level of heavy metal resistance. Screening of the bacterial isolates for metal resistance against Cd^{2+}, Ni^{2+}, Hg^{2+}, Cu^{+2} and Pb^{2+} was done by determining the minimal inhibitory concentration ranging from 10µg/ml to 250µg/ml. All the isolates were screened for their plasmid profile. The size of the isolated plasmid DNA was found to be more than 10,000bp. To determine whether the resistance gene was solely encoded by the plasmid, plasmid curing was done using ethidium bromide. The results showed that the bacterial growth on Cd-supplemented medium was not completely inhibited after plasmid curing, indicating the presence of multimechanisms involved in conferring resistance. It was observed that extracellular polymeric substances produced by isolates MF1 and MF2 play an important role in metal sorption and constitutes a passive method in which the metal cations bind to the negative charges of acidic groups from exopolysaccharide. In the remaining isolates, cadmium is precipitated as cadmium sulfide through hydrogen sulfide production. These heavy metal resistant organisms hold promise for bioremediation of heavy metal polluted environment

Keywords: Bioremediation, heavy metals, metal resistant bacteria, hydrogen sulfide, plasmid

1. Introduction

The growing industrialization has spread worldwide and has left persistent toxic heavy metals, like chromium, nickel, lead, zinc, cadmium and copper in our ecosystem. These heavy metals tend to accumulate and deteriorate the environment. This is especially true for developing countries like China and India (Raja *et al*., 2008). Common sources of heavy metal pollution include discharge from sources such as electroplating, plastic manufacturing industries, fertilizer producing plants and wastes left after mining and metallurgical processes (Zoubonlis *et al*., 2004). The heaviest metals exist naturally in the earth's crust at trace concentrations of just a few parts per million (Bodek *et al*., 1988), sufficient to provide local biota with trace nutrients, but too low to cause toxicity. Disposal of wastes from metal excavation and processing has increased the concentration of these heavy metals to dangerous levels in some soils. Cadmium is a heavy metal recognized as one of the most hazardous environmental pollutants (Lodeiro *et al.*, 2005), which is toxic to humans and aquatic life. Chronic exposure to cadmium can affect the nervous system, liver, cardiovascular system and may lead to renal failure and even death in mammals and humans (Semerjian, 2010). The use of conventional technologies, such as ion exchange, chemical precipitation, reverse osmosis and evaporation recovery for this purpose is often inefficient and or very expensive (Pratik and Hitesh, 2014).The bioremediation of heavy metals using microorganisms is not only a scientific novelty but it is also known for its potential application in industry (Singh *et al.*, 2010). In order to survive in heavy metal polluted environments, many microorganisms have developed a resistance to toxic metal ions (Kumar *et al.*, 2011). Several resistance mechanisms, such as metal efflux, intracellular sequestration by exopolysaccharide cell surface, biosorption by negative groups, bioprecipitation and redox reaction, have been found to be present in microorganisms to counteract heavy metal

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stress (Naik *et al.*, 2012). However, most mechanisms reported involve the efflux of metal ions outside the cell and the genes for tolerance mechanism have been found on both chromosomes and plasmids. Many bacterial strains contain genetic determinants of resistance to heavy metals such as Hg^{2+}, Ag^{2+}, Cu^{2+}, Ni^{2+}, Cd^{2+} and others (Karelova *et al*., 2011). These resistance determinants are often found on plasmids and transposons (Silver and Misra, 1988). The present study was undertaken with the aim of isolating the cadmium resistant bacterial isolates and to study their probable mechanism conferring the resistance. Such microbial populations specifically adapted to high concentrations of heavy metals hold promise for bioremediation of heavy metals from industrial effluents and soil.

2. Material and Methods

2.1. Sample Collection

Soil samples were taken from different locations of battery manufacturing contaminated environment in Faridabad industrial area of Haryana state in India. Soil samples (three to four) were collected randomly from the single site and pooled together. All the samples collected this way from the other sites were stored in sterilized polypropylene bags and kept in the refrigerator at 4^0C.

2.2. Isolation of Cadmium Resistant Bacteria

For isolation of cadmium resistant bacteria, dilution plating was performed; 10-10,000 fold dilutions of fresh soil (1g) were made in sterile distilled water and 0.1 ml from each of these dilutions was placed on Luria Bertani agar (10g peptone, 5g yeast extract, 10g NaCl, 15g agar, pH 7.2) (HiMedia Laboratories Pvt. Ltd.) plates containing 10µg/ml cadmium as cadmium nitrate. The plates were then incubated for 24-48h at 30^0C. Individual colonies of bacteria which varied in shape and color were picked up and further purified. Purified isolates were biochemically characterized as per Bergey's Manual of Systemic Bacteriology.

2.3. Growth Curves of Bacterial Isolates with Metal Induction

An aliquot of 100µl of each bacterial culture was inoculated separately in 100ml of Luria Bertani broth (with and without cadmium nitrate-20µg/ml), and these were incubated at 30^0C in rotary shaker at 500rpm. Aliquots of the culture were taken out in sterilized tubes, at regular intervals of time (6, 12, 24, 48and 72h). Growth was monitored by measuring the Optical Density (OD) at 620nm.

2.4. Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of the metals was determined by plate dilution method as described by Malik and Jaiswal (2000). The metals Cd^{+2}, Pb^{+2}, Hg^{+2} ,$Cu^{+2}$and Ni^{+2} were used as $Cd(NO_3)_2$, $Pb(NO_3)_2$, $HgCl_2$,$CuSO_4$ and $NiSO_4$, respectively. Stocks of the metal salts were prepared in distilled water and sterilized by filter membrane and stored at 4^0C. Luria Bertani medium was prepared and amended with various amounts of Cd, Pb, Hg, Cu and Ni to achieve the desired concentrations of 20, 40, 50, 60, 70, 80, 90, 100, 120, 140, 150,160, 180, 200 and 250µg/ml. Inoculums of all isolates were spread in the metal amended and control plates (without metal). The plates were incubated at 30^0C for 72h. The concentration of the metal, which permitted growth and beyond which there was no growth, was considered as the MIC of the metal against the strain tested.

2.5. Isolation of Plasmid and Plasmid Curing by Ethidium Bromide

The bacterial isolates were screened for the presence of plasmid DNA using the alkaline lysis method (Sambrook and Fritsch, 2001). The isolated plasmid was characterized by agarose gel electrophoresis at 70V for 2h. The gel was stained with ethidium-bromide, visualized under UV transillumination and photographed. For the plasmid curing, twenty four hour old cultures of the isolates were grown in sterile nutrient broth containing ethidium bromide (100µg/ml). The tubes were incubated at 30^0C for 24h. After incubation, the isolates were reinoculated in sterile nutrient broth and incubated further for 24h. The cured isolates were checked for their heavy metal resistance on LB agar plates having different concentrations of the various heavy metals.

2.6. Sulfide Production and Determination of Cadmium Sulfide Precipitation

For the determination of cadmium sulfide production, two experiments were performed. For a simple sulfide detection assay, a semisolid agar medium (Glucose-5.0g, Ammonium phosphate-1.0g, Sodium chloride-5.0g, $MgSO_4.7H_20$, 0.2% Noble agar) containing 2.5mM $FeCl_2.4H_2O$ and 3mM $Na_2S_2O_3.5H_2O$ (HiMedia Laboratories Pvt. Ltd.) was used and observed for the formation of a black precipitate (FeS) in the medium. In another experiment, selected isolates were grown in minimal broth supplemented with 3mm sodium thiosulfate and 50mM $CdCl_2$, incubated at 30^0C without agitation for the observation of orange precipitate.

2.7. Cadmium Accumulation and Removal Assay in the Presence of Thiosulphate

All the isolates were grown in 100 ml LB broth supplemented with 100µg/ml cadmium sulphate and LB broth supplemented with 3mM thiosulfate and 100µg/ml cadmium sulphate. The flasks were incubated on a shaker for 120h at 30^0C. Cells were harvested (One ml) at 24, 36, 48, 72 and 96h of incubation by centrifugation at 1100xg for10minutes at 4^0C. Bacterial cell residue was dissolved in 1ml 95% nitric acid mixed well by vortexing and diluted to 10ml with sterile double distilled water. Blanks were treated in the same way and analyzed by atomic absorption spectrometry. Cadmium was measured from the supernatant by the atomic absorption spectrometry. Percentage of Cd removal by the bacterial cells from the both culture (with and without thiosulfate) was calculated by taking difference between the initial metal content in the culture media and at the time of sampling. Cadmium removal studies were also done under the condition of glucose limitation. The selected isolates were grown in minimal media with and without sodium thiosulphate.

2.8. TEM Analysis

Bacterial cells were grown in media supplemented with cadmium (10µg/ml) for 48h and harvested by centrifugation and

observed in TEM at varying magnifications after treating by the standard methods.

3. Results

Five different bacterial isolates were isolated from the soil samples on LB media supplemented with 10µg/ml of cadmium as Cd $(NO_3)_2$. The morphological and biochemical characteristics of the isolates were studied and results were listed in Table 1. Results showed that two bacterial isolates (MF1 and MF2) were gram positive and three isolates (MF3, MF4 and MF5) were gram negative. Among all, isolates MF1 and MF2 were found to be negative for sulfide production. Growth curves for each of these isolates were studied in the presence of cadmium. Results showed that growth was not considerably affected in the presence of cadmium (Figure 1).

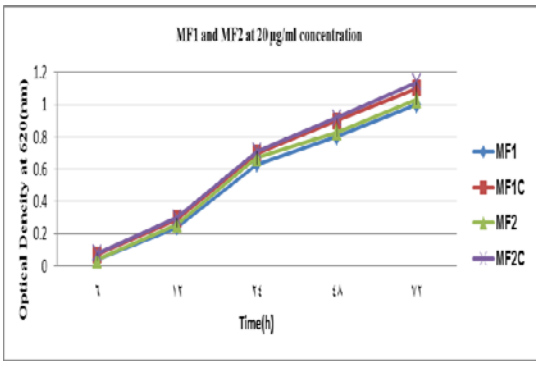
The Minimum Inhibitory Concentration (MIC) of the bacterial isolates was investigated using plate assay to select bacterial isolates capable of growing and tolerating a high level of metal toxicity. The isolates showed a very high degree of resistance to all heavy metals. MIC of cadmium was 100 to 150µg/ml for all the isolates. Minimum Inhibitory Concentration of Pb for all isolates was observed to be 160µg/ml. MIC of Hg was 60 to 80µg/ml for all isolates. MIC of Ni was 50µg/ml for MF1 and MF2, while for the other strains it was 60µg/ml. MIC of Cu was 150µg/mlfor MF4, while for the other strains it was 160µg/ml

(Table 2)). Among the heavy metals, copper and lead were less toxic, whereas nickel and mercury were found to be more toxic to all strains.

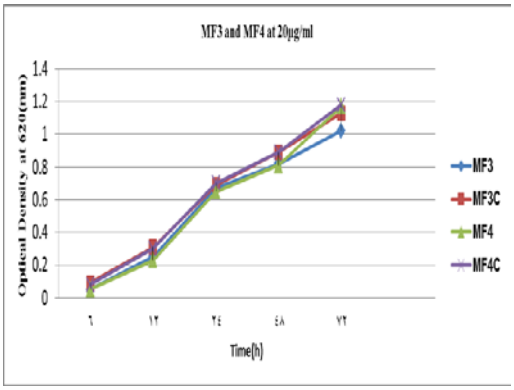
All five bacterial isolates were screened for the presence of plasmid. All the isolates except MF1 showed the presence of a mega plasmid (more than 10,000 bp) (Figure 2). To find out whether the heavy metal resistance gene was plasmid encoded or chromosomal encoded, plasmid curing was carried out by ethidium bromide. Curing results showed that though the growth of the bacteria was retarded on Cd-amended medium after plasmid curing, but it was not completely inhibited (Table 2).

Table 1. Morphological and biochemical Characteristics of the selected isolates

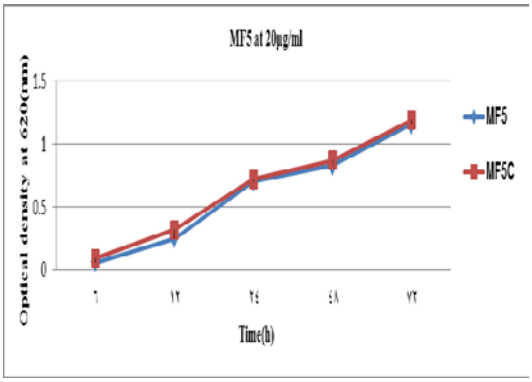
Characteristics	Bacterial isolates				
	MF1	MF2	MF3	MF4	MF5
Gram's reaction	+	+	-	-	-
Colony color	Milky white	white	white dull	Pale yellow	yellow
Spore formation	+	+	-	-	-
Starch hydrolysis	+	+	-	-	-
H_2S production	-	-	+	+	+
Catalase	+	+	-	-	-



(a)



(b)



(c)

Figure1. Growth of isolates (MF1, MF2, MF3, MF4 and MF5) in presence of Cd (20 µg/ml), C= Control

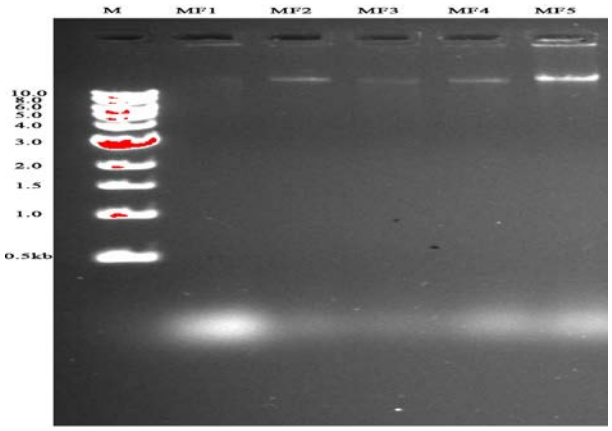


Figure2. Plasmid DNA was extracted and separated by agarose gel electrophoresis. (DNA Marker in lane1).

Table 2. Minimal inhibitory concentrations ppm) of various heavy metals before and after plasmid curing

Bacterial isolates	MIC(ppm)								
	Before Curing				After Curing				
	Cd	Pb	Hg	Cu	Cd	Pb	Hg	Cu	Ni
MF1	100	160	80	160	100	160	80	160	50
MF2	120	160	80	160	60	100	40	100	40
MF3	120	160	60	160	60	100	30	80	40
MF4	140	160	60	160	70	100	30	80	40
MF5	150	160	60	160	80	120	30	100	40

Cadmium removal assay conducted in the presence and absence of sodium thiosulfate showed that there was only 27-35% cadmium removal by isolates MF3, MF4and MF5 as compared to 75-85% by MF1 and MF2 in the absence of sodium thiosulfate. In the presence of sodium thiosulfate, MF3, MF4 and MF5 showed an increase in cadmium removal (75-80%) whereas cadmium removal remains unaffected by MF1 and MF2, after 24h of incubation. TEM analysis showed the entrapment of heavy metal in the EPS (Figure 2).Cadmium removal carried out in minimal media supplemented with 100µg/ml $CdCl_2$ showed a decrease in cadmium removal, while it remains same in isolates MF3, MF4 and MF5 when removal assays are conducted in minimal media supplemented with sodiumthiosulfate (Table 5).

Table 3. Percentage removal of cadmium by bacterial isolates from medium with initial concentration of 100µg/ml cadmium in the presence and absence of Sodium thiosulfate after 24h of incubation at 30^0C.

Bacterial isolates	LB media+100µg/ml$CdCl_2$	LBmedia+3mM $Na_2S_2O_3$+100µg/ml$CdCl_2$
MF1	85	86
MF2	75	77
MF3	29	75
MF4	33	80
MF5	27	77

Table 4 . Cadmium Removal assay under condition of Glucose deficiency.

Heavy metal(µg/ml)	Bacterial isolates				
	MF1	MF2	MF3	MF4	MF5
Cadmium	100	60	40	40	80
Lead	160	100	100	100	120
Mercury	80	40	30	30	30
Copper	160	100	80	80	100
Nickel	50	40	40	40	40

Bacterial isolates	Minimal media+$CdCl_2$		Minimal medium+$CdCl_2$+$Na_2S_2O_3$	
MF1	60	40	70	80
MF2	36	35		
MF3	100	15	70	120
MF4	30	25	77	30
MF5	80	15	62	80

Since the isolates were found to produce hydrogen sulfide, it could be one of the probable mechanisms to provide resistance to cadmium by removing the cadmium as cadmium sulfide. For the determination of cadmium sulfide production, two experiments were performed. For a simple sulfide detection assay, a semisolid agar medium containing 2.5mM $FeCl_2.4H_2O$ and 3mM $Na_2S_2O_3.5H_2O$ was used. The formation of a black precipitate (FeS) in the medium was considered to be an indication of sulfide production. Results showed in Table 4 demonstrate that Cd was efficiently recovered from the solution using bacterial produced H_2S only. The formation of black precipitate was not observed in two isolates (MF1 and MF2). Cadmium removal by bacterial isolates was also investigated in the presence of 3mM $Na_2S_2O_3.5H_2O$ and $CdCl_2$. Among the five, three isolates showed bright yellow precipitate in culture medium after 24h. Results indicate that Cd was precipitated in the form of cadmium sulphide.

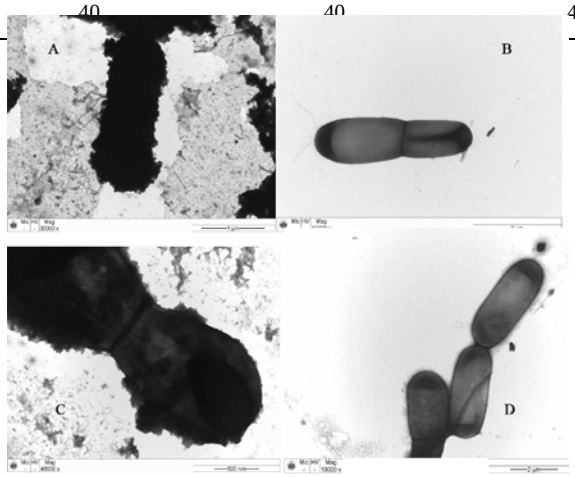


Figure 2. Transmission electron micrographs of isolates MF1 and MF2 (A: MF1grown in the presence of 10µg/ml Cd, B: MF1control, C: MF2 grown in the presence of Cd, D: MF2 control).

4. Discussion

It is very well understood that the environment continuously exposed to heavy metal contaminants favors the growth of bacteria that have developed resistance systems against heavy metal toxicity. Microbial populations in the chronically polluted sites have the capacity to degrade vast range of polluting chemicals. It has been reported that the sites subjected to chronic anthropogenic forces exhibit selection forcatabolically adaptable microbial populations other than the ubiquitous one (Bargiela *et al.*, 2015). In the present investigation, five cadmium resistant bacteria displaying multiple resistances to various heavy metals were isolated from the soils of industrial area, Faridabad. All isolates were able to grow in the presence of 20µg/ml $CdCl_2$ without any significant increase of the lag phase. Similarly results were reported earlier also by Anyanwu and Ugwn (2010).However it has also been reported that at higher concentrations bacteria undergoes physiological adjustment and shows relatively longer lag phase (Krishnamurthy and Rajaram, 2014). All isolates were also found to be resistant to various heavy metals to varying degrees as depicted by their MIC values. The resistance to heavy metals in bacterial strains has been reported to be conferred upon through various mechanisms (Ron *et al.*, 1992). Heavy metals may enter the cell as an alternative substrate for cellular ions transport system. While some bacteria employ mechanisms that cause changes in the transport systems so that the heavy metals no longer enter the cell, others possess several ATPase dependent efflux mechanisms that confer resistance (Tynecka *et al.*, 2016). Precipitation on the cell surface in the form of $CdHPO_4$ and binding of Cd^{2+} by thiols is another mechanism reported in many bacteria (Sinha and Mukharjee, 2009). In another study, Cadmium has been shown to bind to capsular material in *Arthrobacter viscosus* and in *Klebsiella aerogenes* (Hrynkiewicz *et al.*, 2015). A *Citrobacter* mutant isolated from metal-polluted soil was found to accumulate Cd^{2+} as insoluble cell-bound $CdHPO_4$ during growth in the presence of Cd^{2+} and glycerol (Macaskie *et al.*, 1987).

The present work indicates that the isolated bacterial isolates have the ability to resist a wide range of heavy metals. It was observed that all bacterial isolates have multiple heavy metal tolerance and are resistant to Cd, Ni, Pb, Cu and Hg. Resistance to multiple metals has been found in several other bacterial systems and characterized at the molecular level. Liesegang *et al.* (1993) reported that *AlcaligeneseutrophusCH34* harbors numerous heavy metal resistance determinants including three for mercury resistance, one for chromate resistance and two for divalent cations, called czc (for Cd^{2+}, $Zn^{2+}$and Co^{2+}) and cnr (for $Co^{2+}$and Ni^{2+}). Pandit *et al.* (2013) reported that metal resistant bacterial isolates showed high degree of resistance to heavy metals ranging from 25-300ppm. Singh *et al.* (2010) studied that *Pseudomonas aeruginosa* exhibited high resistance to heavy metals with MIC for heavy metals ranging from 50µg/ml to 300µg/ml.

CadA and *cadB* operons represent the two known mechanisms of plasmid-mediated cadmium resistance widespread in bacteria. Bacterial plasmids have genes that confer highly specific resistance to As, Bi, Cd, Cu, Cr, Hg, Zn and other toxic heavy metals. For each toxic cation and anion, generally a different resistance system exists, and these systems may be linked together on multiple resistance plasmids (Silver *et al.*, 1989). In the present study, the plasmid profile of the isolates was found to exhibit a single band indicating the presence of a mega plasmid (more than 10,000bp). Results indicate the presence of plasmid in the selected isolates except MF1. It has been found that large plasmids are responsible for encoding resistance to antibiotics and heavy metals (Jain *et al.*, 2009). Resistance to heavy metals by genes present on their plasmid suggests the extraction of selective pressure on such bacteria through contamination with heavy metals in their environment. Plasmid curing inhibited the growth of all isolates except MF1on media containing cadmium reflecting that the mechanisms involved in conferring resistance are both plasmid as well as chromosomal mediated. Studies have revealed the existence of chromosomal determinants that mediate heavy metal resistance in many organisms. Cad A gene has been found to be a chromosomal determinant in gram positive like *Bacillus subtilis* (Solovieva and Entian, 2004), *Bacillus firmis* (Oger *et al.*, 2003) and gram negative like *Stenotrophomonas maltophilia* (Alonso *et al.*, 2000). Cadmium removal assays showed that there was only 27-33% cadmium removal by isolates MF3, MF4, MF5 as compared to 75-85% byMF1, MF2, reflecting the presence of an active efflux mechanism encoded by cad A operon which although plays an important role in conferring the resistance but is not helpful in the removal of cadmium. They are able to grow in the presence of cadmium but not able to detoxify it. In case of isolatesMF1 and MF2, it was observed that detoxification is achieved by entrapment in the extracellular polymeric substance as determined by TEM. To ascertain the same, the cadmium removal studies were also done under the conditions of glucose limitation under which the production of EPS is greatly inhibited. It was observed that cadmium removal was significantly inhibited in the strains that remove cadmium through EPS and not in strains that precipitate cadmium as cadmium sulfide indicating that biosorption of heavy metals through EPS is one of the mechanisms employed by the isolate used in the present study. Various bacteria have been implicated in removal of heavy metals from industrial wastes and soil through functional groups on their cell envelops (Volesky, 1986; Brierly, 1990). Bacterial extracellular polymeric substances play an important role in metal sorption and constitute a passive method in which the metal cations bind to the negative charges of acidic groups from expolysaccharide.

Since the isolatesMF3, MF4 and MF5 were found to produce hydrogen sulfide, it may also be a putative mechanism for the removal of cadmium in the form of cadmium sulfide. To confirm the formation of cadmium sulfide, the cultures were grown in the broth containing 50mM cadmium chloride and after 24h of incubation a bright yellow colored precipitate was formed due to cadmium sulfide precipitation. Cadmium removal assay was conducted in the medium containing thiosulfate and

it showed 75-86% cadmium removal indicating that one of the mechanisms of resistance to heavy metals is through the production of hydrogen sulfide (H_2S). This production of sulfide might confer cadmium resistance in these isolates for its survival under cadmium stress and it detoxifies cadmium by converting it into insoluble CdS. Many soluble metals can form insoluble complexes with hydroxides, carbonates, phosphates, and sulfides (Gadd and Griffiths, 1978; Fortin *et al.*, 1997). One of the best known natural metal precipitation mechanisms is due to sulfide production H_2S and is produced when sulphur-containing amino acids are decomposed (Valls and Lorenzo, 2002) Microorganisms secrete inorganic metabolic products such as sulphide ions in their respiratory metabolism and with them precipitate toxic metal ions as a form of non-enzymatic detoxification. Metal sulfides possess low solubilities and, therefore, low toxicities because they are biologically unavailable. Many studies have been undertaken with the aim of determining the mechanism of biotransformation of cadmium into cadmium sulfide. *Klebsiealla planticola (Cd-1)* grew anaerobically at a Cd concentration of 15mM and precipitated CdS (Sharma *et al.*, 2000). Bang *et al.* (2002) developed a genetically engineered bacterium capable of producing sulfide under aerobic, microaerobic, or anaerobic conditions for heavy metal precipitation. Microbial population inhabiting polluted sites may have ability to resist much higher concentrations may employ a variety of mechanisms to detoxify the same. As such, efforts need to be directed in revealing and exploiting their real potential. It has been reported that the biodegradation is a process which is mostly performed by the autochthonous bacteria and if environmental condition are optimized using an efficient *ex-situ* treatment such as land farming, such indigenous populations will likely out perform any allochthonous consortium (Fordelianakis *et al.*, 2015). As such, the application of metal-resistant bacteria isolated from the contaminated site for bioremediation offers attractive perspectives. In addition, this approach may also prove to be useful in biological treatment of other organic wastes through protection rendered by the metal accumulating strains to the organic matter degrading bacteria.

5. Conclusion

In the present study, the cadmium resistant bacterial isolates were isolated from the soils collected from Faridabad industrial area. These isolates were found to be resistant to a number of heavy metals besides cadmium. The mechanism involved in conferring resistance to heavy metals was found to be both chromosomal as well as plasmid mediated. Cadmium removal is found to be through both metabolism independent (entrapment in extracellular matrix) and metabolism dependent mechanism (cadmium sulfide production).The detoxification efficiency indicates good potential for application in bioremediation of cadmium from polluted sites. According to the present study, it is very clear that any bacteria that show a fairly good resistance to heavy metals and are capable of producing EPS are potential candidates for heavy metals removal from contaminated site.

References

Alonso A, Sanchez P and Martinez JL. 2000. *Stenotrophomonas maltophilia* D457R contains a cluster of genes from gram-positive bacteria involved in antibiotic and heavy metal resistance. *Antimicrob Agents Chemother,* **44:** 1778-1782.

Anyanwu CU and Ugwn CE. 2010. Incidence of Arsenic resistant Bacteria isolated from sewage treatment plant. *I J Basic Applied Sci,* **10(6):** 43-47.

Bang SW, Clark DS and Keasling JD. 2002. Engineering hydrogen sulfide production and cadmium removal by expression of the thiosulfate reductase gene (phsABC) from *Salmonella enterica serovar typhimurium* in *Escherichia coli*. *Appl Environ Microbio,***66:** 3939- 3944.

Bargiela, *et al.*2015. Bacterial population andbiodegradition potential in chronically crude oil-contaminated marine sediments are strongly linked to temperature. *Scirntific Reports,* **5:** 1-15.

Bodek IB, Lyman WJ, Reehl WF and Rosenblatt DH. 1988. Environmental inorganic chemistry: Properties, processes, and estimation methods. SETAC Spec. Publ. Ser. New York, Pergamon Press.

Brierley JA, 1990. Production and application of a Bacillus-based product for use in metals biosorption. B Volasky (ed.), Biosorption of heavy metals, CRC Press Inc, Boca Raton, Florida, pp-305-312.

Fortin D, Ferris FG and Beveridge TJ. 1997. Surface-mediated mineral development by bacteria. Reviews in *Mineralog Geochem,* **35:** 161–180.

Fodelianakis, *et al.* 2015. Allcothonous bioaugmentation in ex situ treatment of curde oil-polluted in thr presence of an effective degrading indigenousmicrobiome. *J Haz Mat,* **287:** 78-86;

Gadd GM and Griffiths AJ. 1978. Microorganisms and heavy metal toxicity. *Microb Ecol,***4:** 303–317.

Hrynkiewicz K, Zloch M, Kowalkowski T, Baum C, Niedojadlo K and Buszewski B. 2015. Strain-specific bioaccumulation and intracellular distribution of Cd^{2+} in bacteria isolated from the rhizosphere, ectomycorrhizae, and fruitbodies of Ectomycorrhizal fungi. *Pollut Res Int,* **22:** 3055-3067.

Jain PK, Ramachandran S, Shukla V, Bhakuni D, Verma SK. 2009. Characterization of metal and antibiotic resistance in a bacterial population isolated from a Copper mining industry. *Int J Integrated Biology,* **6(2):** 57-61.

Karelova E, Harichova J, Stojnev T, Pangallo D and Ferianc P. 2011. The isolation of heavy-metal resistant culturable bacteria and resistance determinants from a heavy-metal contaminated site, *Biologia,* **66:** 18-26.

Kumar A, Bisht BS and Joshi VD. 2011. Bioremediation potential of three acclimated bacteria with reference to heavy metal removal from waste. *Int J Environ Sci***2(2):** 896-908.

Liesegang H, Lemke K, Siddiqui RA and Schlegel HG. 1993. Characterization of the inducible nickel and cobalt resistance determinant *cnr* from pMOL28 of *Alcaligenes eutrophus CH34*. *J Bacteriol,* **175:** 767-778.

Lodeiro P, Cordero B, Barriada JL, Herrero R and Sastre de Vicente ME. 2005. Biosorption of cadmium by biomass of brown marine macroalgae. *Bioresour.Technol,***96:**1796–1803.

Macaskie LE, Dean ACR, Cheltam AK and Skavnulis AJ. 1987. Cadmium Accumulation by a Citrobacter sp.: the Chemical Nature of the Accumulated Metal Precipitate and its Location on the Bacterial Cells. *Microbiol,* **133(3):** 539-544.

Malik A and Jaiswal R. 2000. Metal resistance in *Pseudomonas* strains isolated from soil treated with industrial wastewater. *World J Microbiol Biotechnol*,**16:**177-182.

Mathivanan K and Rajaram. 2014. Isolation and characterization of cadmium resistant bacteria from an industrially polluted coastal ecosystem on the southeast coast of india. *Chem and Ecolog*, **30(7):** 622-635.

Naik MM, Shamim K and Dubey SK. 2012. Biological characterization of lead-resistant bacteria to explore role of bacterial metallothionein in lead resistance. *Current Sci*, **103(4):** 426-429.

Oger JM, Morel C, Helesbeux JJ, Litaudon M, Seraphin D, Dartiguelonge C, Larcher G, Richomme P and Dural O. 2003. First 2-Hydroxy-3-methylbut-3-enyl substituted xanthones isolated from plants: structure elucidation, synthesis and antifungal activity. *Nat Prod Res*, **17:** 195-199.

Pandit B, Patel PD, Kunjadia and Nagee A. 2013. Isolation, characterization and molecular identification of heavy metal resistant bacteria from industrial effluents, Amala-khadi-Ankleshwar, Gujarat. *I J Environ Sci*, **3(5):** 1689-1699.

Pratik K and Hitesh S. 2014. Screening of Heavy Metal Resistant Bacteria from Nale Lake of Vasai Taluka of Maharashtra. *Int J of Life Sci*, **2(2):** 139-142.

23. Raja S, Ravikrishna, R Yua XY, Leea T, Chenc J, Murugesand K, Xinhua S, Qingzhongc Y, Kalliat T and Collett JL.2008. Fog chemistry in Texas-Louisiana Gulf Coast corridor. *Atmosph Environ J*,**42(9):** 2048-2061.

Ron EZ, Minz D, Finkelstein NP and Rosenberg E. 1992. Interaction of bacteria with cadmium. *Biodegradition*, **3:**161-170.

Sambrook J and Fritsch EF.2001. Molecular cloning-A laboratory manual, third ed. Cold Spring Harbour Laboratory Press, New York, USA.

Semerjian L. 2010.Equilibrium and kinetics of cadmium adsorption from aqueous solutions using untreated *Pinushalepensis* sawdust. *J Hazard Material*,**173:** 236-242.

Sharma PK, Balkwill DL,Frenkel A and Vairavamurthy MA. 2000. A New *Klebsiella planticola* Strain (Cd-1) Grows Anaerobically at High Cadmium Concentrations and Precipitates Cadmium Sulfide. *Appl Environ Microbio*,**66(7):** 3083–3087.

Silver S and Misra TK. 1988. Plasmid-mediated heavy metal resistances. *Annul Rev Microbiol*,**42:** 717-743.

Silver S, Misra TK and Laddaga RA. 1989. DNA sequence analysis of bacterial toxic heavy metal resistances. *Biol Trace Elem Res*,**21:**145-63.

Singh V, Chauhan PK, Kanta R, Dhewa T and Kumar V. 2010. Isolation and Characterization of *Pseudomonas* resistant to Heavy metals contaminants. *Int J Pharmaceutical Sci Review Research*,**3(2):** 164-167.

Sinha S and Mukharjee SK. 2009. *Pseudomonas aeruginosa KUCD1*, A Possible Candidate for Cadmium Bioremediation. *Brazilian. J Microbiol*,**40**: 655-662.

Solovieva IM and Entian KD. 2004. Metalloregulation in *Bacillus subtilis:* the *copZ* chromosomal gene is involved in cadmium resistance. *FEMS Microbiol Letters*, **236:** 115-122.

Tynecka Z, Malm A and Szczsniak ZG. 2016. Cd^{2+} extrusion by P-type Cd^{2+}-ATPase of *Staphylococcus aureus* 17810R via energy-dependent Cd^{2+}/H^{+} exchange mechanism. *Biometals*, **29(4),** 651-663.

Valls M and Lorenzo V. 2002. Exploting the genetic and biochemical capacities of bacteria for the remediation of heavy metal plooution. *FEMS Microbio Reviews*, **26:** 327-338.

VoleskyB. 1986. Biosorbent materials.*Biotechnol bioeng symp*, ***16:****121-126.*

Zouboulis AI, Loukidou MX and Matis KA. 2004. Biosorption of toxic metals from aqueous solutions by bacteria strains isolated from metal-polluted soils.*Proc Bioch*,**39**: 909–916.