Screening and molecular identification of heavy metal resistant *Pseudomonas putida* S4 in tannery effluent wastewater

Wango Nokman, V. Benluvankar, S. Maria Packiam*, S. Vincent

Department of Advanced Zoology & Biotechnology & Loyola Institute of Frontier Energy (LIFE), Loyola College, Chennai, 600 034, India

ABSTRACT

Heavy metal contamination is a serious environmental and ecological problem faced by most of the developing countries including India. The quality of soil and water is highly polluted due to heavy metal pollution therefore, the removal or remediation of heavy metals from the contaminated site has become compulsory. In the current trend, biotechnology provides an opportunity in the removal of heavy metals from a polluted site using microorganisms and microbial products. These technologies provide an alternative and cost-efficient compared to conventional methods. The present study deals with isolation, molecular characterization of heavy metal resistant (Lead [Pb], Chromium [Cr], Silver [Ag], Copper [Co] & Magnesium [Mg]) bacteria from tannery effluent wastewater collected from Vaniyambadi, Tamilnadu. Two isolates were selected based on high level of massive metal resistance. These isolates showed high resistance to heavy metals of lead (Pb) and chromium (Cr) with minimum inhibitory concentration ranging from 800 mg/L to 1800 mg/L. Among this two bacterium isolates LSV (Loyola Savariar Vincent) S4 showed multiple tolerance to various heavy metals. Furthermore, the isolate LSV S4 was susceptible and sensitive to tested antibiotics. The growth kinetics of isolate LSV S4 compared to the control was increased in presence of different heavy metals at regular time intervals. From the basis of morphological, biochemical and 16S rRNA characterization the most potent isolate of LSV S4 was identified as *Pseudomonas putida S4*. The results clearly showed that the novel strain has been effective and highly useful for the bioremediation of heavy metal contaminated environment.

1. Introduction

Heavy metal and their compounds are major environmental pollutants and threat to human life and also affects the soil. Plentiful research has been conducted on heavy metals contamination in water from various sources such as industrial waste, automobile emission, agricultural practices and other anthropogenic activities (Gochfeld, 2003). The excessive level of heavy metals like chromium (Cr), mercury (Hg), silver (Ag), zinc (Zn), lead (Pb) & cadmium (Cd) have no biological role and are harmful to the organisms, even at very low concentrations (Hughes and Poole, 1989; Sinha and Paul, 2014). The need to remediate these natural resources (soil, water & air) has led to the development of new technologies that emphasize the destruction of the pollutants rather than the conventional approach of disposal because of their potential to enter the food chain (Asha and Sandeep, 2013). The removal of heavy metals from the environment is a major environmental concern and its removal can be done by biotic methods and abiotic methods include physiochemical processes whereas it's very expensive and generate secondary products (Celis et al., 2000). There

are many reports says that the biological processes are considered cost-effective and are environmentally friendly methods for the removal of heavy metal contaminated effluent (Pandit et al., 2013). Microbial activity plays a vital role to remove different concentration of heavy metals and often or specific to a single molecule or a few metals (Silver and Mistra, 1988; Silver and Phung, 1996; Majáre and Bülow, 2001; Nies, 2003; Piddock, 2006). The mechanism of heavy metal degradation through microorganisms includes biosorption, bioleaching, biomineralization, immobilization and redox reactions (Lloyd, 2002). Biosorption is the most common mechanism for heavy metal detoxification based on energy requirements (Haferburg and Knothe, 2007). Several authors have been reported that the capacity of surviving and the ability of heavy metal tolerant bacteria was isolated by various sources (Basu et al., 1997; Choudhury and Kumar 1998; Castro-Silva et al., 2003; Otth et al., 2005; Lima-Bittencourt et al., 2007; Othman and Ahamad, 2015). Diverse microbes have been proposed to be an efficient and economical alternative in the removal of heavy metals from water (Waisberg et al., 2003). This current work aimed to characterize and determine the heavy metal resistant and antibiotic

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* Corresponding author.
  E-mail address: smariap@gmail.com (S. Maria Packiam).

sensitive pattern of a new bacterial strain isolated from tannery effluent.

2. Materials and methods

2.1. Sample collection

The wastewater sample was collected from tannery effluent around Vaniyambadi, Tamil Nadu (India). Wastewater samples were taken in a sterile plastic container and transported to the laboratory in aseptic condition.

2.2. Isolation and screening of heavy metal resistant bacteria

A serial dilution of the sample (1 ml of water sample) was made using sterile saline until a dilution of 10^{-6} was obtained. 100 μl of this dilution was spread over nutrient agar Petri plates supplemented with heavy metals like lead in lead acetate and chromium in potassium dichromate. The concentration of each heavy metal was 100 mg/L and it was incubated at 37 °C for 24 h. Distinct colonies that appeared on these selective media were isolated and subcultured in the same medium at 37 °C. The culture was streaked and kept in an incubator at 37 °C for 24 h and was preserved in slants under a refrigerator.

2.3. Determination of minimum inhibitory concentrations (MICs)

Minimum Inhibitory Concentrations (MICs) of the heavy metal resistant bacterial isolate grown on heavy metals supplemented media against respective heavy metal were determined by gradually increasing the concentration of metal salts on nutrient agar plates until the complete inhibition growth on the plates. The culture growing on the first concentration (100 mg/L) was transferred to the higher concentration such as 200, 400, 600, 800, 1000 and 1200 mg/L by streaking on the plates. MIC was noted when the isolates failed to grow on the plates, even after 48 h of incubation.

2.4. Determination of Co-resistance to other heavy metals

The isolated heavy metal resistant strain was tested for their resistance to the test of the other heavy metal such as mercury, silver and copper. The concentration of the heavy metals in this test was 200 mg/L and 400 mg/L.

2.5. Determination of antibiotic sensitivity and resistance pattern

The antibiotic sensitivity test was determined by well diffusion method on Muller Hinton Agar plates. The antibiotics like Ampicillin (20 μg), Streptomycin (20 μg), Nalidixic acid (20 μg), Penicillin (20 μg) were placed on the agar plates and incubated at 37 °C for 24 h. The Zone of inhibition was measured.

2.6. Growth kinetics of heavy metal resistant strain in presence of heavy metals

The growth kinetics of heavy metal resistant strain was studied in Nutrient Broth medium in the presence of Chromium (400 μg/ml) and lead (800 μg/ml) was added in the broth. The growth pattern of the strain was identified by analyzing the absorbance value at 600 nm at different time intervals. The bacterial culture without the addition of heavy metals were considered as control.

2.7. Identification and characterization of heavy metal resistant bacteria

Heavy Metal resistant bacteria was characterized by the shape of the cell, color, gram stain, motility and biochemical analysis using Indole Test, Catalase, Oxidase, Methyl Red Test, Voges-Proskauer Test, Citrate Utilization Test, Catalase Test, Urease Test and Amylase Test (according to the Bergey's Manual of Systematic Bacteriology).

2.8. Isolation of genomic DNA and 16SrRNA gene amplification

Genomic DNA was isolated using Nucleospin Tissue Kit (Macherey-Nagel) following manufactures instructions. Bacterial 16SrRNA gene was amplified by using the universal bacterial primers, F (5′ CAG GCC TAA CAC ATG CAA GTC – 3′) and R (5′ GGG CGG WGT GTA CAA GGC-3′). PCR (Polymerase Chain Reaction) was performed with 20 μl reaction mixture containing 1X PCR buffer (100mM Tris HCl, pH-8.3; 500mM KCl), 0.2mM each dNTPs (dATP, dGTP, dCTP and dTTP), 2.5mM MgCl₂, 1 unit of AmpliTaq Gold DNA polymerase enzyme, 0.1 mg/ml BSA, 4% DMSO, 5pM of forward and reverse primers and template DNA. After the initial denaturation for 5 min at 95 °C for 30 s, annealing at 60 °C for 1 min, extension at 72 °C for 1 min and final extension at 72 °C for 7 min. PCR products were analyzed by 1.2% (w/v) agarose gel electrophoresis in 0.5X TBE buffer with Ethidium bromide (0.5 μg/ml).

2.9. Phylogeny

The removal of unwanted primers and dNTPs from PCR products of 16S rRNA genes of isolates treated with ExoSAP-IT (Affymetrix USB) consist of two hydrolytic enzymes (Exonuclease I and Shrimp Alkaline Phosphatase in buffer). The purified PCR Products of 16S rRNA was mixed with using Big Dye Terminator v3.1 Cycle sequencing kit (Applied Biosystems, USA) and then sequenced. Sequences were matched with previously published bacterial 16S rRNA sequences in the NCBI databases using BLAST.

Based on the scoring index the most similar sequences were aligned with the sequences of other representative bacterial 16S rRNA regions using Clustal X software (version 1.83). Further phylogenetic tree information and similarity index were generated and compared with known sequences. The 16S rRNA sequences of bacterial isolates were deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases with accession number is KX721487.

3. Results

3.1. Isolation and screening of heavy metals resistant bacteria

In the present study, we identify and characterize heavy metal-resistant bacteria isolated from tannery effluent wastewater. The isolated colonies were picked up from the plates according to their different form and purified by sub-culturing on the nutrient agar plates using the streak plate method. Twelve colonies were screened from the initial level of heavy metal supplemented NA medium. Only two bacterial strains were shown resistance to tested heavy metal (Chromium & Lead) concentration 200 mg/L. The heavy metal resistant isolate called as isolate LSV S3 (Fig. 1) and LSV S4 (Fig. 2), which were resistant to

Fig. 1. Isolate LSV S3.

Fig. 2. Isolate LSV S4.

chromium and lead.

3.2. Minimum inhibitory concentration (MIC) of heavy metals

The minimum inhibitory of concentration (MIC) of tannery effluent isolate LSV S3 and LSV S4 against heavy metals such as chromium and lead was determined in solid media ranged from 200 mg/L to 2000 mg/L. Bacterial isolate LSV S4 showed a very high degree of resistance to lead and the MIC value is 1600 mg/L. However, the same isolate showed activity against chromium is less and the value is 1000 mg/L. The isolate LSV S3 showed very less activity compared to isolate LSV S4 and there is no much more growth in the concentration of 600 mg/L of lead and 400 mg/L of chromium heavy metals (Table 1).

3.3. Co-resistance to other metals

Bacterial isolate LSV S4 co-resistance to silver and copper up to 400 mg/L whereas, the isolate LSV S3 was not able to resist the silver and copper even 200 mg/L. There is no growth was observed in both cultures were streaked on mercuric chloride containing plates. Finally, the isolate LSV S4 was selected based on a high degree of heavy metal resistance and it was used for further studies (Table 2).

3.4. Antibiotic susceptibility test

The bacterial isolate LSV S4 was found to be susceptible to all the tested antibiotics. The maximum inhibition of 33.73 ± 0.40 mm against Nalidixic acid and the minimum zone of inhibition was 9.23 ± 0.46 to Ampicillin shown in Table 3. In general, the isolate exhibits maximum susceptibility to Nalidixic acid followed by Penicillin, Streptomycin and Ampicillin.

Table 1
Minimum inhibitory concentrations of heavy metals.

Concentrations (mg/L)	Isolate LSV S3		Isolate LSV S4	
	Lead	Chromium	Lead	Chromium
200	+ + +	+ + +	+ + +	+ + +
400	+ + +	+ +	+ + +	+ + +
600	+	–	+ + +	+ + +
800	–	–	+ + +	+ +
1000	–	–	+ + +	+
1200	–	–	+ +	–
1400	–	–	+ +	–
1600	–	–	+	–
1800	–	–	–	–
2000	–	–	–	–
MIC (mg/L)	600	400	1600	1000

(+, Very less; + +, Moderate; + + +, High growth; -, No growth).

Table 2
Co-resistance to other Metal.

Concentrations (mg/L)	Isolates LSV S3			Isolate LSV S4		
	Ag^{2+}	Co^{2+}	Mg^{2+}	Ag^{2+}	Co^{2+}	Mg^{2+}
200	–	–	–	+	+	–
400	–	–	–	+	+	–

(+, growth; -, no growth).

Table 3
Antibiotic Susceptibility Test of isolate LSV S4.

Isolate LSV S4	
Antibiotics concentration (20µg)	Mean Value
Ampicillin	9.23 ± 0.46
Streptomycin	24.13 ± 0.30
Nalidixic acid	33.73 ± 0.40
Penicillin	16.84 ± 0.15

Values are given as means $\pm$ SD (n = 3).

3.5. Growth kinetics of the bacterial isolate in presence of heavy metals

The isolate LSV S4 exhibited different growth patterns in the presence of different heavy metals. An increased growth was observed in isolate LSV S4 inoculated in lead compared to control and it followed by very less growth in chromium at regular interval times. The growth curve of isolate LSV S4 similar to the typical bacterial growth curve over the experiment period. The experiment showed that isolate LSV S4 increased their growth and reached to maximum growth at 48 h. The absorbance value at 600 nm of isolate LSV S4 in presence of heavy metals at different time interval is plotted in Fig. 4.

3.6. Identification of microbial isolate

The most potent isolate LSV S4 was identified and characterized. The isolate was gram-negative, motile and rod-shaped cells. The morphological and biochemical characteristic results showed in that the bacterial isolate LSV S4 were related to the members of *Pseudomonas* sp. (Table 4)
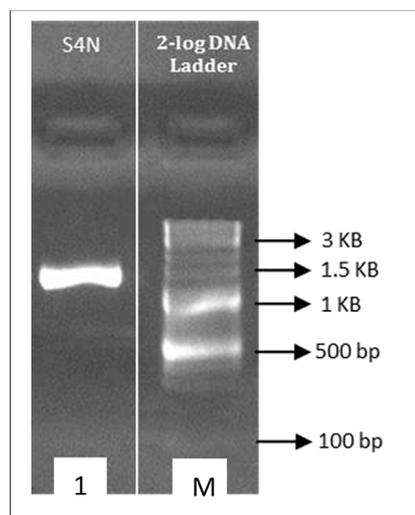

Fig. 3. PCR amplification of Isolate LSV S4. Agarose gel (1%) electrophoresis of PCR products of 16S rRNA gene isolates of LSV S4: (Lane 1) and Marker (M) is represented (Lane 2).
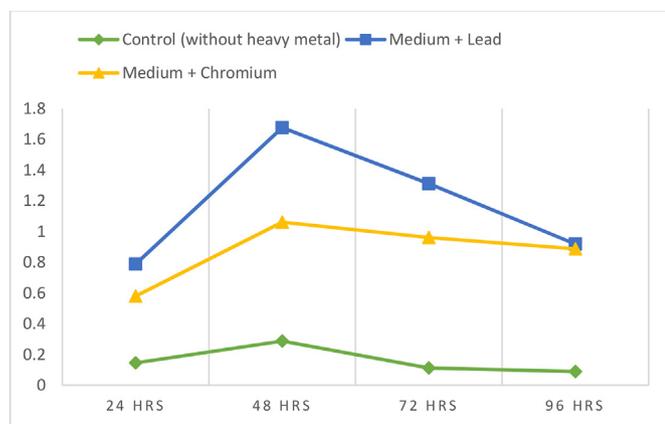
Fig. 4. Kinetics of isolate in presence of heavy metals. Growth curve of isolate LSV S4 (*Pseudomonas putida* S4) in the presence of heavy metals.

Table 4
Biochemical Characterization of isolate LSV S4.

S.No	Characteristics	Results
1.	Cell Morphology	Rod-shaped
2.	Colony Color	Whitish & cream
3.	Gram Stain	Negative
4.	Motility	Positive
5.	Indole	Negative
6.	Methyl Red	Negative
7.	Catalase	Positive
8.	Oxidase	Positive
9.	Voges Proskauer	Negative
10.	Citrate Utilization	Positive
11.	Urease	Positive
12.	Starch Hydrolysis	Negative
13.	Casein Hydrolysis	Positive
14.	Gelatin Hydrolysis	Negative
15.	High salt tolerance	Positive

3.7. Molecular characterization of the most potent bacterial isolate

The most potent isolate LSV S4 further characterized by PCR amplification of the 16S rRNA gene produced fragments of approximately 1500 base pairs in size (Fig. 3). The PCR products were sequenced and the resulted sequences compared with available sequences in the database. The results indicated the most significant similarity to members of the *Pseudomonas* group, which matched the conclusions of the morphological and biochemical analysis. Finally, the isolate LSV S4 was identified as *Pseudomonas putida* S4 (GenBank accession number - KX721487) (Fig. 5).
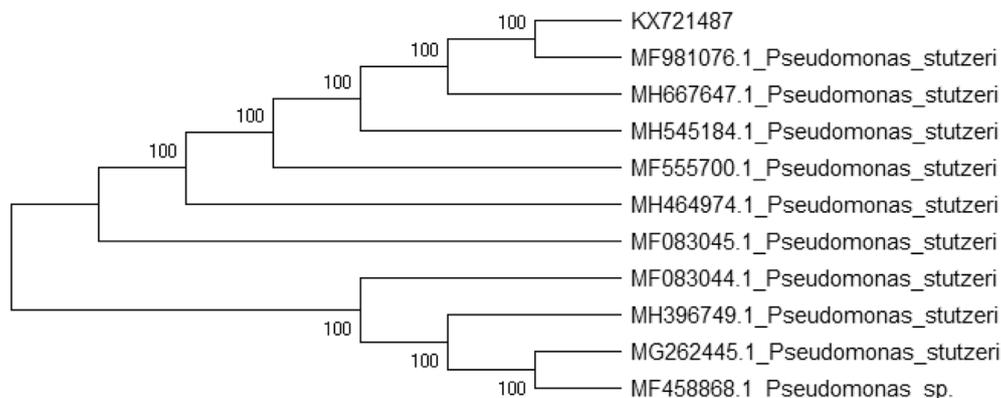
4. Discussion

Water pollution due to toxic heavy metals through tannery effluent remains a severe environmental and public problem in developing countries. Bioremediation can be a reliable and cost-efficient process for removing heavy metals from the polluted site (Dias et al., 2002; Khan and Jaffar, 2002). There is a need for an investigation on the isolation and characterization of the bacteria with metal tolerance to identify potential isolate for heavy metal bioremediation (Abo-Amer et al., 2014; Nwagwu et al., 2017). In the present study, we identified and characterized heavy metal resistant bacteria isolated from tannery effluent water. Twelve isolates were screened from an initial level of heavy metal supplemented NA medium. Only two bacterial isolates from tannery effluent water samples showed resistance to heavy metals concentration 200 mg/L that was tested. Previous results reported that fifty isolates of heavy metal resistant bacteria isolated at the initial level of medium supplemented with heavy metal from sewage wastewater and nine isolates exhibited their heavy metal resistance to tested heavy metals of concentration 800–1200 mg/ml. These include Cd (Cadmium) 1-1, Ag1-1, Ag1-2, Ag1-3, Ag1-4, Sn (Tin) 1-1, Sn1-2, Sn1-3 and Sn1-4, which were resistant to Cd, Ag. Twenty bacterial isolates were obtained from Lead (Pb) supplemented LB Medium using tannery effluent collected from Chittagong city, Bangladesh (Marzan et al., 2017). However, our results reported that the minimum inhibitory concentrations (MICs) of isolates LSV S3 was 600 mg/L for lead and 400 mg/L for chromium, isolate LSV S4 against the heavy metals ranged from 1600 mg/L for lead and 1000 mg/L for chromium. Khusro et al. (2014) reported that, the *Bacillus subtilis* strain KPA can tolerate chromium at 1000 mg/L. Our findings are more or less similar to their investigation. Ahmed et al. (2005) reported that bacteria has the ability to adapt in presence of high levels of heavy metals in their environment by developing various resistance mechanisms. These mechanisms could be helpful for the removal of heavy metals from the contaminated site. The bacterial isolate *Alcaligenes faecalis* from sewage wastewater showed high resistance to heavy metals with minimum inhibitory concentrations (MICs) for heavy metal ranging from 800 to 1400 mg/ml (Abo-Amer et al., 2015). In our study isolate LSV S4 showed multiple tolerance to heavy metals except for Mg^{2+} among other tested and isolate LSV S3 did not show any tolerance ability after increasing heavy metal concentration of 400 mg/L. Multiple tolerance happens due to variations in resisting mechanism and cell wall composition of the heavy metal resistant bacteria (Shruti et al., 2012). The recent study reported that *Alcaligenes faecalis* isolated from sewage wastewater has the ability to co-resistance to Cd^{2+}, Pb^{2+}, Ag^{2+} and Al^{3+} (Abo-Amer et al., 2015). Marzan et al. (2017) also reported that multiple tolerance ability of *Gemella* sp. against to Cd - 1350 µg/L, Cr - 360 µg/L as well as Pb - 1900 µg/L. Khusro et al. (2014) reported that *Bacillus subtilis* strain KPA has the ability to tolerate and grow at different concentrations of

Fig. 5. Phylogenetic Analysis of Isolate LSV S4. Phylogenetic tree of bacterial isolate LSV S4 based on the nucleotide sequences of the 16S rRNA genes. The GenBank accession number of the bacteria is presented as KX721487.

chromium. We found that, our results are more or less similar to aforementioned investigations. Strain LSV S4 was not able to tolerate Mg^{2+} due to degrees of poly metabolic activity. The bacterial strain LSV S4 showed tolerance to some of the heavy metals tested at different antibiotics such as ampicillin, streptomycin, nalidixic acid and penicillin at 20 µg/ml concentration. The high range of susceptibility was found in nalidixic acid with 33.3 mm zone of inhibition. Many investigations are reported the antibiotic susceptibility of heavy metal resistant bacteria (Mgbemena et al., 2012; Tamtam et al., 2011). In the study by Owolabi and Hekeu (2014) found that the heavy metal bacterial isolate of *Pseudomonas* sp. showed 62% resistance to 13 antibiotics tested *Streptococcus* showed 38% and *Corynebacterium* sp. showed 69%. Due to various resistant factors were helps the bacteria to adapt themselves in high levels of metals and antibiotics. The growth kinetics of isolate LSV S4 in presence of heavy metals indicated that growth of the heavy metals stressed bacteria was found to be increased compared to the control till 96th hour. Interestingly isolate LSV S4 resistance and tolerance capacity to Pb, lead and the optical density (lambda (λ) - 600 nm) were higher than compared to control. Similar results have been reported earlier (Edward Raja et al., 2009; Abo-Amer et al., 2015; Marzan et al., 2017). The bacterial isolate LSV S4 were then characterized by morphological and biochemical methods. Depending on gram staining the isolate LSV S4 as gram-negative bacteria by detecting peptidoglycan layer which is present in a thick layer in bacteria (Burke and Pister, 1986). The molecular characterization of isolate was done and the phylogenetic tree of heavy metal resistant bacterial isolate LSV S4 constructed based on the nucleotide sequences of the 16S rRNA genes. The isolate was identified as *Pseudomonas putida* S4 and its GenBank accession number is KX721487. The present study recapitulates that the most potent heavy metal resistant bacteria were used to detoxify the heavy metal contaminated tannery effluents and this isolate can be a promising candidate for wastewater treatment technology.

5. Conclusion

Discharge of industrial waste has resulted in the accumulation of heavy metals in wastewater. Therefore, the heavy metal resistant bacteria could be a potential agent for bioremediation of heavy metal. From the present study, it is clear that heavy metal resistance bacterial strains effectively removed the heavy metals in laboratory experiments. Twelve bacterial strains were isolated from sewage water by using an enrichment isolation technique based on a high-level heavy metal resistance of chromium. The bacterial isolate S4 showed high resistance to heavy metals with MICs ranging from 200 mg/L to 2000 mg/L. The effective isolate was characterized and identified as *Pseudomonas putida* S4 (GenBank accession number - KX721487). Therefore, the ability of heavy metal resistant bacterial isolate would be helpful in wastewater treatment.

Acknowledgments

References

Abo-Amer, A.E., Abu-Gharbia, M.A., Soltan, E.M., Abd El- Raheem, W.M., 2014. Isolation and molecular characterization of heavy metal-resistant *Azotobacter chroococcum* from agricultural soil and their potential application in bioremediation. Geomicrobiol. J. 31, 551–561.

Abo-Amer, Aly, E., El-Shanshoury, Abd El-Raheem, R., Alzahrani, Othman M., 2015. Isolation and molecular characterization of heavy metal-resistant *Alcaligenes faecalis*

from sewage wastewater and synthesis of silver nanoparticles. Geomicrobiol. J. 32, 836–845.

Ahmed, N., Nawaz, A., Badar, U., 2005. Screening of copper tolerant bacterial species and their potential to remove copper from the environment. Bull. Environ. Contam. Toxicol. 74, 219–226.

Asha, L.P., Sandeep, R.S., 2013. Review on bioremediation – potential tool for removing environmental pollution. Intern. J. Basic Appl. Chem. Sci. 3, 21–33.

Basu, M., Bhattacharya, S., Paul, A.K., 1997. Isolation and characterization of chromium-resistant bacteria from tannery effluents. Bull. Environ. Contam. Toxicol. 58, 535–542.

Burke, Pister, 1986. Cadmium transport by a Cd^{2+} ⁻ sensitive and a Cd^{2+} ⁻ resistant strain *of Bacillus subtilis*. Can. J. Microbiol. 32, 539–542.

Castro-Silva, M.A., Souza Lima, A.O., Gerchenski, A.V., Jaques, D.B., Rodrigues, A.L., Lima de Souza, P., Rörig, L.R., 2003. Heavy metal resistance of microorganisms isolated from coal mining environments of Santa Catarina. Braz. J. Microbiol. 34, 45–47.

Celis, R., Hermosin, C.M., Cornejo, J., 2000. Heavy metal adsorption by functionalized clays. Environ. Sci. Technol. 34, 4593–4599.

Choudhury, P., Kumar, R., 1998. Multidrug and metal-resistant strains of *Klebsiella pneumoniae* isolated from *Penaeus monodon* of the coastal waters of deltaic Sundarban. Can. J. Microbiol. 44, 186–189.

Dias, M.A., Lacerda, I.C., Pimentel, P.F., de Castro, H.F., Rosa, C.A., 2002. Removal of heavy metals by an *Aspergillus terreus* strain immobilized in a polyurethane matrix. Lett. Appl. Microbiol. 34, 46–50.

Gochfeld, M., 2003. Cases of mercury exposure, bioavailability, and absorption. Ecotoxical. Environ. Saf. 56, 174–179.

Haferburg, G., Kothe, E., 2007. Microbes and metals: interactions in the environment. J. Basic Microbiol. 47, 453–467.

Hughes, M.N., Poole, R.K., 1989. Metals and Microorganisms. Chapman and Hall, London.

Khan, A., Jaffar, M., 2002. Lead contamination of air, soil and water in the vicinity of Rawal Lake, Islamabad. Pakistan J. Appl. Sci. 2, 816–819.

Khusro, A., Preetam Raj, J.P., Panicker, S.G., 2014. Multiple heavy metals response and antibiotic sensitivity pattern of *Bacillus subtilis* strain KPA. J. Chem. Pharmaceut. Res. 6, 532–538.

Lima-Bittencourt, C.I., Cursino, L., Gonçalves-Dornelas, H., Pontes, D.S., Nardi, R.M.D., Callisto, M., Chartone-Souza, E., Nascimento, A.M.A., 2007. Multiple antimicrobial resistance in Enterobacteriaceae isolates from pristine freshwater. Genet. Mol. Res. 6, 510–521.

Lloyd, J.R., 2002. Bioremediation of metals: the application of microorganisms that make and break minerals. Microbiol. Today 29, 67–69.

Majáre, M., Bülow, L., 2001. Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals. Trends Biotechnol 19, 67–73.

Marzan, L.W., Hossain, M., Mina, S.A., Akter, Y., 2017. Isolation and biochemical characterization of heavy-metal resistant bacteria from tannery effluent in Chittagong city, Bangladesh: bioremediation viewpoint. Egypt. J. Aqua. Res. 43, 65–73.

Mgbemena, C.I., Nnokwe, J.C., Adjeroh, L.A., Onyemekara, N.N., 2012. Resistance of bacteria isolated from Otamiri River to heavy metals and some selected antibiotics. Curr. Res. J. Biol. Sci. 4, 551–556.

Nies, D.H., 2003. Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiol. Rev. 27, 313–339.

Nwagwu, E.C., Yilwa, V.M., Egbe, N.E., Onwumere, G.B., 2017. Isolation and characterization of heavy metal tolerant bacteria from Panteka stream, Kaduna, Nigeria and their potential for bioremediation. Afr. J. Biotechnol. 16, 32–40.

Othman, M.A., Ahamed, N.T., 2015. Isolation and characterization of heavy metal resistant *Bacillus subtilis* spp. collected from water sources of taif province of Saudi arabia. Int. J. Curr. Microbiol. App. Sci. 4, 350–357.

Otth, L., Solís, G., Wilson, M., Fernández, H., 2005. Susceptibility of *Arcobacter butzleri* to heavy metals. Braz. J. Microbiol. 36, 286–288.

Owolabi, J.B., Hekeu, M.M., 2014. Heavy metal resistance and antibiotic susceptibility pattern of bacteria isolated from selected polluted soils in lagos and ota, Nigeria. Int. J. Basic Appl. Sci. 14, 6–12.

Pandit, R., Patel, B., Kunjadia, P., Nagee, A., 2013. Isolation, characterization and molecular identification of heavy metal resistant bacteria from industrial effluents, Amala-khadi Ankleshwar. Gujarat. Inter. J. Environ. Sci. 3, 1689–1699.

Piddock, L.J., 2006. Multidrug-resistance efflux pumps not just for resistance. Nat. Rev. Microbiol. 4, 629–636.

Raja, E.C., Selvam, G.S., Omine, K., 2009. Isolation, identification and characterization of heavy metal resistant bacteria from sewage. In: International Joint Symposium on Geodisaster Prevention and Geoenvironment in Asia. JS-Fukuoka, pp. 205–211.

Shruti, M., Bali, G., Saranya, S.K., 2012. Lead biosorption by a bacterium isolated from industrial effluents. Int. J. Microbiol. Res. 4, 196–200.

Silver, S., Mistra, T.K., 1988. Plasmid mediated metals resistance. Annu. Rev. Microbiol. 42, 717–743.

Silver, S., Phung, L.T., 1996. Bacterial heavy metal resistance: new surprises. Annu. Rev. Microbiol. 50, 753–789.

Sinha, S.N., Paul, D., 2014. Heavy metal tolerance and accumulation by bacterial strains isolated from wastewater. J. Chem. Biol. Phys. Sci. 4, 812–817.

Tamtam, F., Van Oort, F., Lebot, B., Dinh, T., Mompelat, S., Chevreuil, M., Lamy, L., Thiry, M., 2011. Assessing the fate of antibiotic contaminants in metal contaminated soils four years after cessation of long term waste water irrigation. Sci. Total Environ. 409, 540–547.

Waisberg, M., Joseph, P., Hale, B., Beyersmann, D., 2003. Molecular and cellular mechanism of cadmium carcinogenesis. Toxicology 192, 95–117.