International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 3 Number 10 (October-2015) pp. 26-34

www.ijcrar.com

Screening and Optimization of Heavy Metal Resistance in *Alcaligenes faecalis* Isolated from Contaminated Sites of Chambal Region-First Report

Akanksha Gupta, Raghvendra Saxena and Vivek Bajpai*

Department of Life Sciences, SOLS, ITM University-Gwalior (M.P) 474001

Corresponding author

KEYWORDS

Heavy metal tolerance bacteria, 16S rDNA

A B S T R A C T

Heavy metal pollution increases day by day due to the intensive discharge of hazardous waste in soil and ground water. This hazardous waste generated by the activities of industries i.e. metal processing, surface treatment, smelting and mining, and the uncontrolled dumping of waste in soil. Living microorganisms used to reduce the contamination of heavy metal and waste by the process known as bioremediation. In the present study heavy metal resistant bacteria were isolated from soil of Chambal region. Three isolates were selected and characterized on the basis of morphological, biochemical and molecular (16s rDNA) characters. The isolates were identified as *Alcaligenes faecalis* (AG-3), *E. coli* (AG-5) and *Pseudomonas aeruginosa* (AG- 8). The temperature, pH and heavy metal tolerance were optimized, the screened bacterial isolates shown highest growth at 30^{o}C±2 and pH 7.0±0.2 and resistance against cadmium chloride, lead acetate, nickel chloride, mercury and cesium chloride. Results revealed the high resistance efficiency of isolates against various environmental factors and heavy metals.

Introduction

A pollutant is a harmful substance in the environment, which causes objectionable effects, impairing the welfare of the environment. Compromises the quality of life and may ultimately cause death. Such a substance has to be present in the environment away from a set or tolerance limit, which could be either a required or acceptable limit. Environment is highly polluted by heavy metal, Wastewaters from the industries and sewage sludge applications have permanent toxic effects to human and the surrounding environment (Rehman *et al.*, 2008). Due to the rapid globalization and urbanization contribute environment pollutants by toxic heavy metal. The main sources of pollution particularly by heavy metals are usually linked with areas of intensive industry and heavy automobile use. Various approaches have been used to detoxify and clean up these metals, such as the use of certain

chemicals which in turn, cause secondary pollution and physical methods that require large input of energy and are very expensive materials, However, microbes have evolved mechanisms to tolerate the occurrence of heavy metals either by efflux, complexation or reduction of metal ions or to use them as terminal electron acceptors in anaerobic respiration process (Gadd, 1992; Nies and Silver, 1995). The important toxic metal pollutants like cadmium, nickel and lead enter to the water bodies through industrial wastewater treatment plants (Denise *et al.*, 1989; Ajmal *et al.*, 1998). Cadmium (Cd) is nonessential but poisonous for plants, animals, and humans (Gupta and Gupta, 1998). 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 industries, incineration of plastics and batteries, land application of sewage sludge, and burning of fossil fuels (Tang *et al.*, 2006). High concentrations of cadmium is highly corrosion resistant and is widely used to plate metal parts in general industrial hardware as well as in automobiles, electronics, marine and aerospace industries (Herrero *et al.,* 2005). Removal of excesses of heavy metal ions from wastewaters is essential due to their extreme toxicity towards aquatic life and humans. Lead (Pb) a major pollutant that is found in soil, water and air is a hazardous waste and is highly toxic to human, animals, plants and microbes (Low *et al.,* 2000). Nickel (Ni) is the 24th most abundant element in the earth crust and has been detected in different media in all parts of the biosphere. Ni is classified as the borderline metal ion because it has both soft and hard metal properties and can bind to sulfur, nitrogen and oxygen groups (Costa and Klein, 1999). Removal of toxic heavy metals from contaminated industrial sites is essential from the environmental point of view to combat pollution. The study tends to determine the resistance pattern of bacterial strains isolated from Chambal region to Heavy metal.

Human activities such as mining operations and the discharge of industrial wastes have resulted in the accumulation of metals in the environment and eventually are accumulated through the food chain, leading to serious ecological and health problems (Gupta and Kumar, 2012).

Heavy metals are the main threat for human health. Heavy metal intoxications may damage central nervous function, the cardiovascular and gastrointestinal (GI) systems, lungs, kidneys, liver, endocrine glands, and bones. Chronic heavy metal exposure has been implicated in several degenerative diseases of these same systems and may increase the risk of some cancers. (Lakherwal, 2014). Metal resistant microbes develop the mechanisms which help in detoxification or cleaning-up of the metal from that environment. This possible environmental application to remove dangerous heavy metal from the contaminated soil and its characterization to explore it further for its suitability for bioremediation of heavy metal contaminated (Joshi and Modi, 2013). These bacteria possessing plasmids carry the genes for metal resistance, and since such traits are plasmid borne, they can be easily transmitted from one bacterium to an entire population. Such metal tolerable bacteria are now favored for their capacity to depollute the environment and hence regarded as instruments for bioremediation (Jasmine *et al.,* 2012). Cadmium is the most dangerous and harmful metal ion characterized by high stability and toxicity. It is not degradable in nature and will thus, once released to the environment, stay in circulation. Cadmium is known to bind with essential respiratory

enzymes (Nies, 2003) causing oxidative stress and cancer (Banjerdkji *et al.,* 2005).

Materials and Methods

Sample collection

The areas under study for this work were identified based on the need, diversity and extent of pollutants produced by various industries located in Gwalior, Madhya Pradesh. Samples were collected from the soil of four different sites, which are waste dumping area and metal dumping area at Chambal Region, Gwalior. 20 samples were collected at distinct places within a particular site were transported in sterile bags/bottles to the Microbiology laboratory, School of Life Science, ITM. University - Gwalior for further analysis.

Isolation of bacteria from soil

One gram of soil was suspended in 10 ml sterilized distilled water and serial dilution were done to obtain dilutions up to 10^{-7}. One ml suspension of each 10^{-1} to 10^{-7} dilutions of the sample was separately plated with nutrient agar medium and incubated at 37°C for 48 hrs. Specific bacterial colonies were selected and isolated by repeated streaking on the nutrient agar medium at 37^{o}C, to obtain pure culture at every 48hrs interval.

Identification of bacterial isolates

The bacterial isolates were subjected to various tests from the study of their morphology and growth pattern on different agar media, different microbiological identification tests such as gram staining and motility tests followed by biochemical identification tests like catalase, citrate utilization, urease, indole production, hydrogen sulphide production, nitrate and nitrite reduction, methyl red, Voges Proskeur and sugar fermentation tests. Molecular identification was also done as per standard protocol.

Molecular identification

On the basis of morphological, microbiological and biochemical identification, three isolates were selected for Molecular characterization. Genomic DNA was extracted by CTAB protocol. In brief, bacterial cells were collected by centrifugation at 13,000 rpm for 2 min followed by suspension in 564µl Tris-HCl-EDTA buffer and incubation with 20µl lysozyme (100mg/ml) at 37°C for 30 mins. 40 µl SDS (10%) were added mixed well and 8 µl proteinase K (10mg/ml) were mixed and incubated for 1 hour at 37^{o}C. Lysis solution, 100µl of 5M NaCl was added and incubated for 2 min at 65^{o}C. This was followed by an addition of 100µl CTAB/NaCl and a further incubated for 10 min at 65^{o}C. The mixture was treated with phenol/chloroform/isoamyl alcohol (25:24:1). The supernatant was collected and precipitated with isopropanol by keeping at – 20^{o}C overnight. Genomic DNA was washed in 70% ethanol and dissolved in 100ml TE buffer. RNase treatment was carried out to remove traces of RNA from the sample.

Bacterial 16S rDNA was amplified by using the universal bacterial 16S rDNA primers, F (5 ’- AGA GTT TGA TCC TGG CTC AG – 3’) and R (5’- GGT GTT TGA TTG TTA CGA CTT - 3’). PCR was performed The PCR (50ml) contained 0.5ml of each forward and reverse primer, 1.5mM of 10X Taq buffer (stock 20mM), 0.125 mM (2.5ml) of each deoxynucleotide (ddATP, ddGTP, ddCTP and ddTTP), 1.25 units of Taq DNA polymerase. PCR conditions were as follows: denaturation at 94^{o}C for 3 min, 30 cycles of denaturation at 95^{o}C for 1 min,

annealing at 55°C for 1 min, extension at 72°C for 1 min, and a final extension step at 72°C for 10 min. The PCR products obtained from DNA extracted from the samples were first analyzed by electrophoresis in 1.5% agarose gel and was stained with ethidium bromide and visualized under short-wavelength UV light.

16S rDNA sequencing of the isolated strain was done. Sequences obtained were compared to the non-redundant nucleotide database at the National Center for Biotechnology Information by using their World Wide Website and the BLAST (Basic Local Alignment Search Tool) algorithm.

Determination of optimal growth conditions

The optimal growth conditions with reference to pH and temperature were determined. The isolates were grown in nutrient broth medium with different pH values (5, 6, 7, 8, and 9) and incubation was carried out at temperature 20°C, 30°C, 37°C and 50°C. The optical density of the log phase culture (8–10 h) was noted at 650nm to determine the growth.

Determination of optimum heavy metal resistance of isolated strains

Isolates were also screened for their ability to resist against various heavy metals and their different concentration. Cells of overnight grown cultures were inoculated on Luria Bertani (LB) broth supplemented with different concentrations (10, 20, 30, 40 and 50 µg/ml) of heavy metals (lead in lead nitrate, cadmium in cadmium chloride monohydrate, cesium in cesium chloride, nickel in nickel chloride). The inoculated Luria Bertani broth without the addition of heavy metals serves as the control. These cultures were incubated at 37°C in an incubator shaker at 30 rpm for 7 days and OD was observed at every 24hrs interval to obtain standard growth curve for absorbance versus time. The effects of different concentrations of heavy metal ions on growth were noted.

Results and Discussion

Isolation of heavy metal resistant bacteria

In the present study we identify and characterize heavy metal resistant bacteria isolates from polluted soil of Chambal region. Three hundred colonies were screened from initial level of heavy metal supplemented LB medium. Twenty isolates were selected in the secondary screening. One isolate was selected for study based on high level of heavy metal resistances.

Morphological and biochemical characteristics of the bacterial isolates

On the basis of Morphological and Biochemical study, the isolate AG-3 was identified as *Alcaligenes faecalis*, a gram negative rod and found positive for catalase test and sugar fermentation (Table 1).

Molecular identification

Comparative analysis of the sequences of AG 3 with already available database showed that the strains were closed to the members of genus *Alcaligenes.*

Determination of the effect of heavy metals on bacterial growth: Growth pattern with 10µg/ml to 50µg/ml of heavy metal against *Alcaligenes faecalis*

The growth pattern of the *Alcaligenes faecalis* with the concentration of 10µg/ml to 50µg/ml of various heavy metals (Lead, Cadmium, Cesium and Nickel) is given in table 2 and figure 1 to 5.

Table.1 Morphological and biochemical identification of *Alcaligenes faecalis*

Bacterial Strain	**Gram Staining/ Shape**	**Biochemical Test**									
		Glucose fermentation	Sucrose fermentation	Fructose fermentation	Indole	Nitrate reductase	H_2S	MR	VP	Citrate	Catalase
AG -3 *(Alcaligenes faecalis)*	-ve/rod	Y	Y	Y	--	-	-	-	-	-	+

Figure.1 Growth pattern of *Alcaligenes faecalis* against different heavy metal at concentration of 10µg/ml

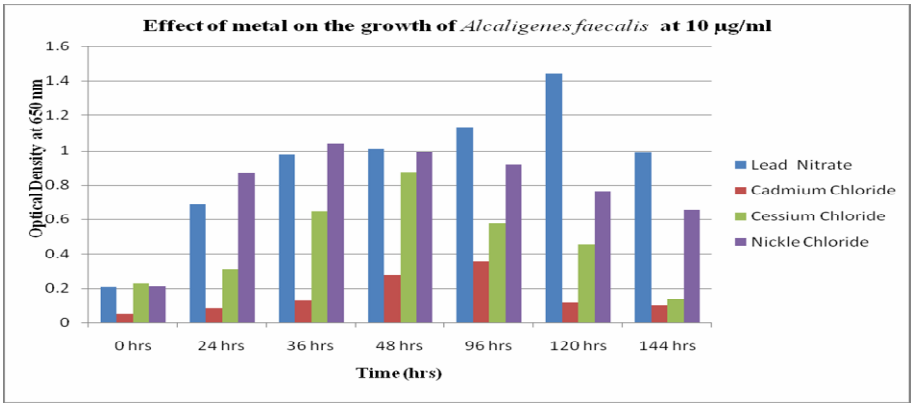


Figure.2 Growth pattern of *Alcaligenes faecalis* against different heavy metal at 20µg/ml concentration

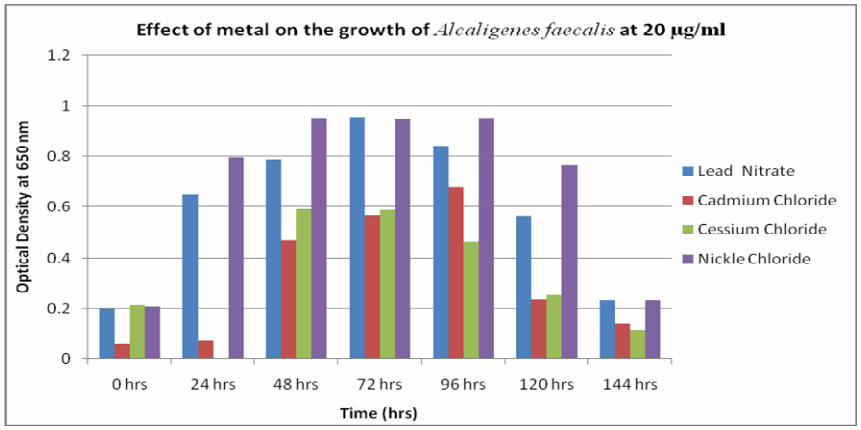


Table.2 The growth pattern of the *Alcaligenes faecalis* with the various concentration of heavy metals (Lead, Cadmium, Cesium and Nickel)

Time/Heavy metal	Lead Nitrate	Cadmium Chloride	Cesium Chloride	Nickel Chloride
10µg/ml				
0 hrs	0.2055	0.053	0.228	0.212
24 hrs	0.69	0.085	0.312	0.872
48 hrs	0.977	0.127	0.649	1.038
72 hrs	1.01	0.28	0.876	0.992
96 hrs	1.13	0.354	0.576	0.919
120 hrs	1.44	0.114	0.453	0.765
144 hrs	0.986	0.099	0.134	0.657
20µg/ml				
0 hrs	0.198	0.061	0.214	0.209
24 hrs	0.652	0.075	0.408	0.797
48 hrs	0.788	0.47	0.593	0.954
72 hrs	0.958	0.569	0.588	0.951
96 hrs	0.844	0.678	0.462	0.955
120 hrs	0.566	0.235	0.256	0.768
144 hrs	0.234	0.1375	0.114	0.234
30µg/ml				
0 hrs	0.212	0.062	0.186	0.256
24 hrs	0.581	0.0835	0.224	0.987
48 hrs	1.108	0.44	0.394	1.045
72 hrs	0.863	0.614	0.355	0.867
96 hrs	0.852	0.564	0.241	0.923
120 hrs	0.675	0.245	0.233	0.657
144 hrs	0.453	0.1345	0.112	0.256
40µg/ml				
0 hrs	0.217	0.096	0.182	0.289
24 hrs	0.489	0.0565	0.209	0.997
48 hrs	0.603	0.766	0.407	1.054
72 hrs	0.678	0.8325	0.366	0.851
96 hrs	0.733	0.534	0.213	0.755
120 hrs	0.544	0.324	0.2	0.512
144 hrs	0.234	0.2105	0.171	0.344
50µg/ml				
0 hrs	0.227	0.092	0.149	0.237
24 hrs	0.477	0.1125	0.216	0.345
48 hrs	0.527	0.5465	0.266	0.982
72 hrs	0.534	0.44	0.257	0.678
96 hrs	0.728	0.344	0.324	0.453
120 hrs	0.545	0.231	0.112	0.469
144 hrs	0.237	0.1155	0.088	0.129

Figure.3 Growth pattern of *Alcaligenes faecalis* against different heavy metal at 30µg/ml concentration

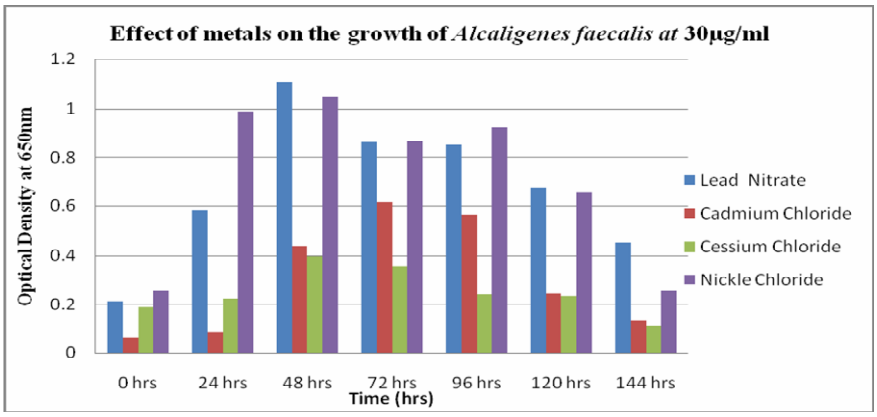


Figure.4 Growth pattern of *Alcaligenes faecalis* against different heavy metal at concentration of 40µg/ml

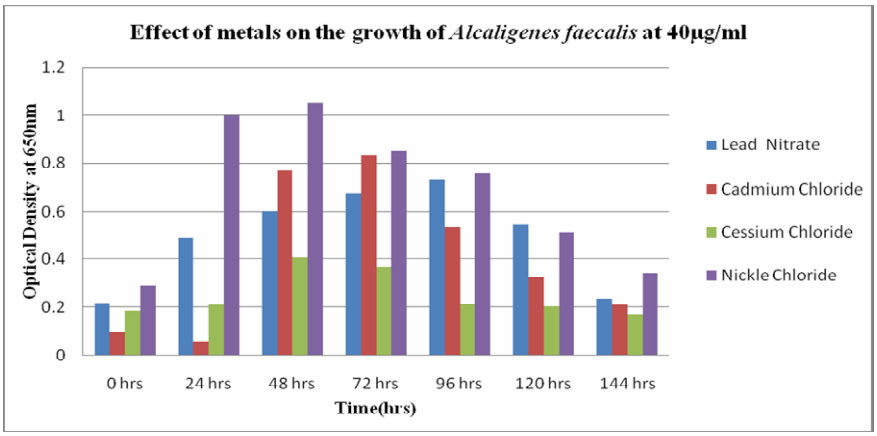
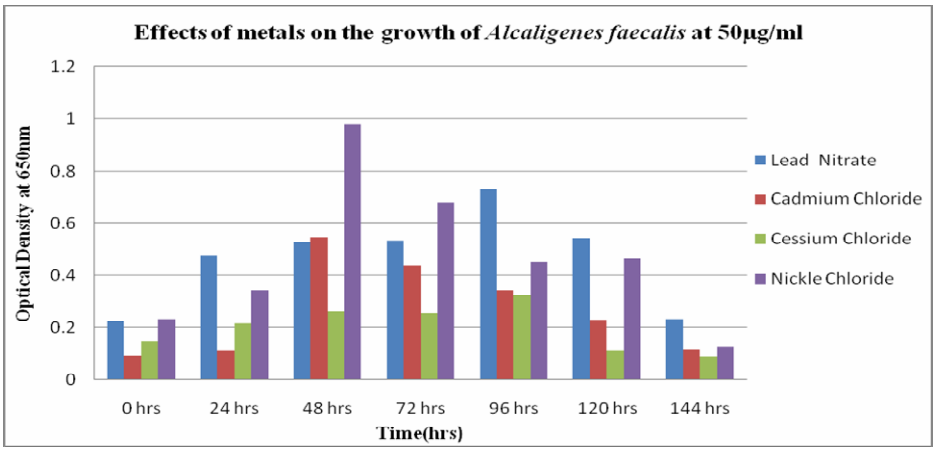


Figure.5 Growth pattern of *Alcaligenes faecalis* against different heavy metal at concentration of 50µg/ml



Present study was based on the toxic nature of Metals against bacterial growth which can be used as inhibitor against the pathogenic culture of bacteria and can be used to improve the effect of microbial biofertilizer especially in the fields where metal concentration is more than permitted limit.

During present study the identified culture is reported as *Alcaligenes faecalis which is* morphologically gram negative rod and is able to ferment Glucose, Sucrose and Fructose.

The most effective metal which is able to inhibit the growth of *Alcaligenes faecalis is* cesium chloride and cadmium chloride whereas Nickel has shown least effect on the growth of *Alcaligenes faecalis* at all concentrations. Lead nitrate is also showing less inhibiting properties. Present study is important because it is providing a method to inhibit the growth of non desirable bacteria but also it is providing the information regarding toxicity of various metals.

References

Ajmal, M., Mohammad, A., Yousuf, R., Ahmad, A. 1998. Adsorption behaviour of cadmium, zinc, nickel and lead from aqueous solution by *Mangifera indica* seed shell. *Indian J. Environ. Health*, 40(1): 5–26.

Banjerdkji, P., Vattanaviboon, P., Mongkolsuk, S. 2005. Exposure to cadmium elevates expression of genes in the *oxy R* and *Ohr R* regulons and induces cross-resistance to peroxide killing treatment in *Xanthomona scampestris*. *Appl. Environ. Microbiol.*, 71(4): 1843–1849.

Costa, M., Klein, C.B. 1999. Nickel carcinogenesis, mutation, epigenetics, or selection. *Environ. Health Perspect. Part A*, 107: 438–439.

Denise, P., Higham, P., Sadler, J., Michael, D. 1989. Cadmium resistance in *Pseudumonas putida*: growth and uptake of cadmium. *J. Gen. Microbiol.*, 131: 2539–2544.

Gadd, G.M. 1992. Metals and microorganisms: A problem of definition. *FEMS Microbiol. Lett.*, 100: 197–204.

Gupta, N., Kumar, V. 2012. Identification and isolation of heavy metal (copper) resistant bacteria. *Scholars Res. Libr. Arch. Appl. Sci. Res.*, 4(1): 577–583.

Gupta, U.C., Gupta, S.C. 1998. Trace element toxicity relationships to crop production and livestock and human health: implications for management. *Commun. Soil Sci. Plant Anal.*, 29: 1491–1522.

Herrero, R., Lodeiro, P., Rey-Castro, C., Vilariño, T., Sastre de Vicente, M.E. 2005. Removal of inorganic mercury from aqueous solutions by biomass of the marine macroalga *Cystoseira baccata*. *Water Res.*, 39: 3199–3210.

Jasmine, R., Venkadesan, B., Ragul, K. 2012. Identification and characterization of heavy metal-resistant *Pseudomonas aeruginosa* and its potential for bioremediation. *Am. J. Pharm. Tech. Res.*, 2(4).

Joshi, B.H., Modi, K.G. 2013. Screening and characterization of heavy metal resistant bacteria for its prospects in bioremediation of contaminated soil. *J. Environ. Res. Dev.*, 7(4A): 1531.

Lakherwal, D. 2014. Adsorption of heavy metals, a review. *Int. J. Environ. Res. Dev.*, 4(1): 41–48.

Low, K.S. Lee, C.K., Liew, S.C. 2000. Sorption of cadmium and lead from aqueous solution by spent grain. *Proc. Biochem.*, 36(1): 59–64.

Nies, D.H. 2003. Efflux mediated heavy metal resistance in prokaryotes. *FEMS. Microbiol. Rev.,* 27(2-3): 313–39.

Nies, D.H., Silver, S. 1995. Ion efflux systems involved in bacterial metal resistances. *J. Indian Microbiol.,* 14: 186–199.

Rehman, A., Zahoor, A., Muneer, B., Hasnain, S. 2008. Chromium tolerance and reduction potential of a *Bacillus* sp.ev3 isolated from metal contaminated wastewater. *Bull. Environ. Contam. Toxicol.,* 81: 25–29.

Tang, X.Y., Zhu, Y.G., Cui, Y.S., Duan, J., Tang, L. 2006. The effect of ageing on the bioaccessibility and fractionation of cadmium in some typical soils of China. *Environ. Int.,* 32: 682–689.