Pak. J. Chem. 7(1-4): 12-16, 2017 Full Paper
ISSN (Print): 2220-2625
ISSN (Online): 2222-307X DOI: 10.15228/2017.v07.i01-4.p03

Heavy Metal Resistant Bacteria: A Potential Candidate for Bioremediation

S. A. Hassan[1]*, E. Hanif[1], A. Siddiqui[1], E. Asif[1], U. H. Khan[1],
A. K. Tanoli[2]
[1]Department of Biotechnology, University of Karachi, 75270, Karachi.
[2]Department of Chemistry, University of Karachi, 75270, Karachi.
*email of corresponding author: shafaqah@uok.edu.pk

ABSTRACT:

Microorganisms have numerous biotechnological applications for bioremediation of metal-contaminated soil and water systems. In view of this, the present study was aimed to evaluate the abilities of microorganisms for tolerance and biosorption of heavy metals. In this study we have isolated 30 different bacterial strains from soil. Morphological and biochemical characterization was carried out. It was shown that *Pseudomonas* sp. was most common isolate among heavy metal resistant organisms that are capable of resisting 5 heavy metals (Ni, Pb, Cr, Cu and Cd) of concentration range 50-300µg/ml. Among 30 isolates, 4 of them showed high degree of resistance against all metals at concentration at 300µg/ml. Bacterial strains isolated in this study can be used in bioremediation and bio-mining.

Keywords: Metal Resistance, Bioremediation, Maximum Tolerance Concentration MTC.

1. INTRODUCTION

Heavy metals have adversed affect on the environment and living organisms [1]. Industrial effluents containing heavy metals are reported to pose health hazards to plants, animals and humans [2]. The commonly encountered heavy metals in environment include a numerous metal ions like arsenic, cadmium, chromium, copper, lead, nickel and zinc are of immense threat for overall environment [3]. Heavy metals may enter the environment by man-made activities. Soil erosion, mining, industrial effluents, urban runoff, sewage discharge are among potential sources of these contaminants [4].

Heavy metals are known to have their affect on cell organelles like cell membrane, mitochondrial, lysosome, endoplasmic reticulum, involved in metabolism, detoxification, damage repair in biological systems. Metal ions interact with DNA and nuclear proteins, causing DNA damage and cause conformational changes that may lead to cell cycle modulation, carcinogenesis or apoptosis [5]. Detoxification of contaminated site with the help of microorganism has evolved as the most effective tool to combat the problem of pollution. Microorganisms are being exploited for their ability to remediate the toxic pollutants released in the environment due to human activities and industrialization [6]. Biosorption, extracellular precipitation, accumulation of metals, oxidation-reduction and efflux of heavy metals from the cell are among the several mechanisms developed by microorganisms to resist heavy metals as reported by many researchers [7-9]. The interest in heavy metal uptake by bacteria has been increased nowadays especially because of their biotechnological potential that microorganisms have for the removal and recovery of metals [10]. Bacteria are known as good biosorbents and with the proper understanding may be in near future. They proved to be good alternatives for the removal of metal ions from the environment [10]. The present study was aimed to isolate and screened the heavy metal resistant bacterial strains from the soil that can be exploit in future for bio-mining and bioremediation.

2. MATERIALS AND METHODS:

2.1. Materials:

Microbiological culture media used in this study was purchased from Oxoid ltd. All the salts reagents and chemical used in this study were purchased from Merck chemicals and were of analytical grade.

2.2. Sample Collection

The soil sample was collected from road side (University road, Karachi) in ziplock bags and processed in Department of Biotechnology, University of Karachi.

*Corresponding Author Received 01th April 2017, Accepted 15th June 2017

2.3. Isolation of Metal Resistant Bacteria:

To isolate bacteria, nutrient agar plates were prepared and supplemented with appropriate volume of metal salt solutions. For initial screening of metal resistant bacteria, the saline dilution was made and added to nutrient agar plate containing metal salts and then incubated overnight at 37°C. Isolated colonies were taken from nutrient agar and inoculated in nutrient broth and nutrient agar slants.

2.4. Preservation of Bacterial Strains:

Isolated strains of bacteria were preserved on agar slants at 4°C and in 80% glycerol at -20°C.

2.5. Identification of bacteria

Isolated bacteria were identified by biochemical characterization as described by Bergey's Manual [11]. The test includes gram staining, motility test, catalase test, coagulase test, oxidase test, indole test, citrate test and urease test were performed.

2.6. Screening of metal resistant bacteria

Screening of isolated colonies against metal ions was done by increasing the concentration by factor 50. Nutrient agar media was prepared with different concentrations of metal salts. Metal resistant test was performed against metal i.e. Nickel (Ni), Lead (Pb), Chromium (Cr), Copper (Cu) and Cadmium (Cd). The growth in plates was then examined and minimum inhibitory concentration was calculated.

3. RESULTS:

3.1. Isolation of Microorganism

Samples were collected from roadside area of University road, Gulshan e Iqbal, Karachi. A total of 30 microorganisms were isolated, which were preserved on agar slants and in glycerol for further studies.

3.2. Identification of Bacteria

The isolates were identified using Bergey's Manual of Systematic Bacteriology [11]. Based on colonial morphology, Gram's stain and biochemical characteristics, 12 isolates were identified as *Pseudomonas* sp., 5 were identified as *Rhodococcus* sp., 2 were identified as *Staphylococcus* sp., 3 were identified as *Proteus* sp., 6 were *Bacillus* sp., and 1 isolate of *Micrococcus* sp. and *Campylobacter* sp. each respectively. The results are summarized in Table 1.

Table 1: Identification of Bacterial strains

S. No	Bacteria	No of Isolates
1	*Pseudomonas* sp	12
2	*Bacillus* sp	6
3	*Rhodococcus* sp	5
4	*Proteus* sp	3
5	*Staphylococcus* sp	2
6	*Micrococcus* sp.	1
7	*Campylobactor* sp.	1

3.3. Maximum Tolerance Concentration:

To evaluate the maximum tolerance concentration, the isolated bacterial strains were tested against different concentrations of metal salts i.e. 50 µg/ml, 100 µg/ml, 150 µg/ml, 200µg/ml, 250 µg/ml and 300 µg/ml for their metal resistance. The data obtained from results is shown in Figure 1-3. Among the resistant isolated bacteria, 4 isolates found to be resistant against all metal ions at all concentrations studied.

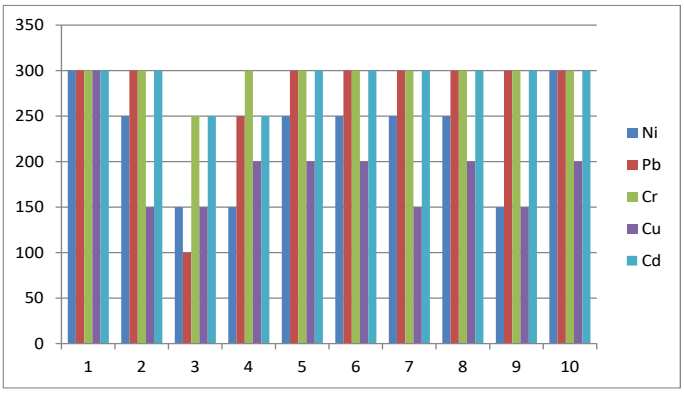

Figure 1. The Maximum Tolerance Concentration (MTC) of isolates 1-10

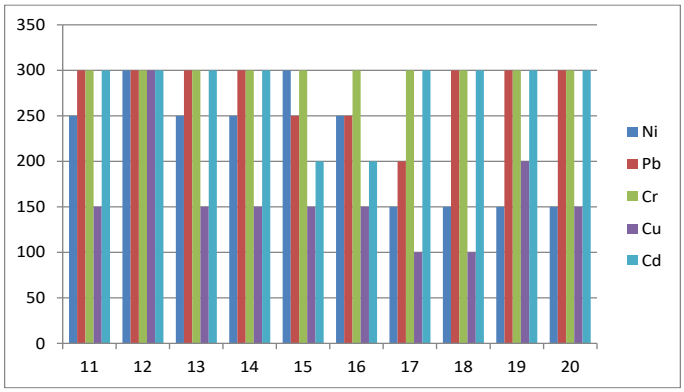

Figure 2. The Maximum Tolerance Concentration (MTC) of isolates 11-20

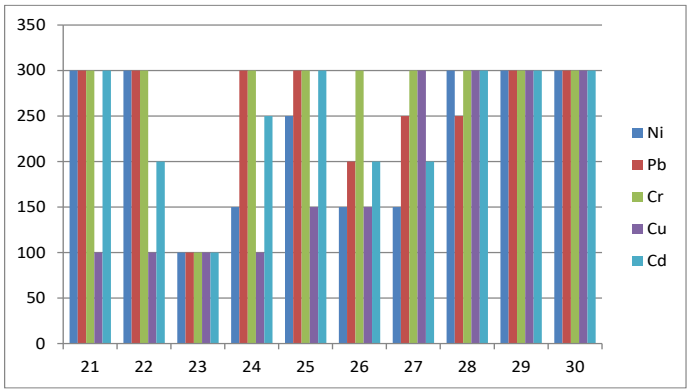

Figure 3. The Maximum Tolerance Concentration (MTC) of isolates 21-30

4. DISCUSSION:

Bacteria are exposed to heavy metals in their growth environment so they have evolved to this stress by developing resistance to these notorious compounds. These resistance processes evolved by bacteria could be explored for detoxification of heavy metals in various cleanup experiments. In most of the reports, metal resistance has been found to be associated with antibiotic resistance also [12].

In the field of bioremediation, simpler and relatively economical ways are more attractive than chemical and physical treatment methods. Furthermore, many investigators have reported the bioaccumulation of Cadmium onto natural microbial populations like bacteria and algae as the new bioremediation technology [13]. The microorganism uses various survival strategies to combat the Cadmium stress that include metal ions sequestration, active efflux of metals, some use enzymatic detoxification and Cadmium accumulation [14]. In this study, 21 out of 30 isolates showed resistance at 300µg/ml. Reduction of Chromium Cr^{6+} has been reported by many researchers in various different bacterial species such as *Desulfovibrio* sp. [14], *Microbacterium* sp. [15] and *shewanella* sp. [16]. The availability of an effective bacterial strain is an essential requirement for the development of bioremediation process aimed for detoxification of contaminated site. In our study, 24 isolates were highly resistant to chromium at 300µg/ml concentration. Researchers have been used lead-resistant microorganisms for the immobilization and biosorption of lead [17]. Lead resistance was found in 20 out of 30 isolates. Singh et al. [18] (2010) also reported gram negative isolates showing resistance with maximum tolerable concentration (MTC) for nickel ion. In the present study, nickel resistance was found to be low as only 4 strains were resistant to high concentration studied. Altimira et al. [19] isolated five copper-resistant species that were resistant to high Cu^{+2} concentration ($\geq$ 2.8 mM) and other heavy metals. Out of 30 isolates, 5 were resistant to copper at high concentrations.

The capabilities of microorganisms to detoxify the effects of heavy metals or to detect heavy metals are to be effectively explored in environmental biotechnology. Physico-chemical methods used for the remediation of heavy metal contaminated soil could damage its structure and quality, as well as existing ecosystems. Additionally, these techniques require soil excavation and transport to mending sites, and this causes high costs and the requirement of specialized equipments. According to the results present in this study suggests that the identified bacteria could be used in bioremediation of heavy metal contaminated sites and in biominig. The ability of microbial strains to grow in the presence of heavy metals would be helpful in the treatment of contaminated sites such as beaches and dump sites where microorganisms are being involved in the decomposition of toxic compounds.

REFERENCES:

[1]. L. Järup; *Br. Med. Bull.*, 68, 167-182. **(2003)**.

[2]. R. A. Bernhoft; *J. Environ. Public Health*, (**2012)**.

[3]. M. Lambert, B. Leven, & R. Green; *Environmental science and technology briefs for citizens. Kansas State University, Manhattan, KS*. **(2000).**

[4]. S. Morais, F. G. e Costa, & M. de Lourdes Pereira; *In Environmental health-emerging issues and practice*, IntechOpen, **(2012)**.

[5]. M. P. Rayman; *The lancet*, 356, 233-241, **(2000)**.

[6]. C. Garbisu, & I.Alkorta; *Bioresour. Technol.*, 77, 229-236, **(2001)**.

[7]. F. François, C. Lombard, J. M. Guigner, P. Soreau, F. Brian-Jaisson, G. Martino, M. Vandervennet, D. Garcia, A. L. Molinier, & D. Pignol; *Appl. Environ. Microbiol.*, 78, 1097-1106, **(2012)**.

[8]. K. Hrynkiewicz, & C. Baum; *In Environmental deterioration and human health*, 215-227: Springer. **(2014)**.

[9]. C. M. Monteiro, P. M. Castro, & F. X. Malcata; *Biotechnol. Prog.*, 28, 299-311, **(2012)**.

[10]. M. Valls, & V. De Lorenzo; *FEMS Microbiol. Rev.*, 26, 327-338, **(2002)**.

[11]. P. H. Sneath, N. S. Mair, M. E. Sharpe, J.G. Holt. Bergey's manual of systematic bacteriology. Volume 2: Williams & Wilkins; (**1986)**.

[12]. S. Verma, & R. Dubey; *Biol. Plant.*, 44, 117-123, **(2001)**.

[13]. M. Rafatullah, O. Sulaiman, R. Hashim, & A. Ahmad, *Wood Sci. Technol.*, 46, 221-241, **(2012)**.

[14]. S. Sinha, & S. K. Mukherjee; *Braz. J. Microbiol.*, 40, 655-662, **(2009)**.

[15]. P., Pattanapipitpaisal, N., Brown, & L. Macaskie; *Biotechnol. Lett.*, 23, 61-65, **(2001)**.

[16]. C. Myers, B. Carstens, W. Antholine, & J. Myers, *J. Appl. Microbiol.*, 88, 98-106, **(2000)**.

[17]. H. J. Park, G. Gusarova, Z. Wang, J. R. Carr, J. Li, K. H. Kim, J. Qiu, Y. D. Park, P. R. Williamson, & N. Hay; *EMBO Mol. Med.,* 3, 21-34, **(2011)**.

[18]. V. Singh, P. Chauhan, R. Kanta, T. Dhewa, & V. Kumar; *Int. J. Pharm. Sci. Rev. Res.*, 3, 164, **(2010)**.

[19]. F. Altimira, , C. Yáñez, G. Bravo, M. González, L. A. Rojas, & M. Seeger; *BMC Microbiol.*, 12, 193, **(2012).**