EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 8
501-505
Year: 2018

HEAVY METAL TOLERANCE OF SOIL BACTERIA ISOLATED FROM PLANT RHIZOSPHERE REGION OF *VIGNA RADIATA*, L.

Prasanti Devi, Rasmita Padhy, S. P. Nayak, S. Panigrahi and B. K. Mohanty®

P.G. Department of Botany and Biotechnology, Khallikote Autonomous College, Berhampur-760001, Odisha, India.

***Corresponding Author: B. K. Mohanty**
P.G. Department of Botany and Biotechnology, Khallikote Autonomous College, Berhampur-760001, Odisha, India.

Article Received on 16/06/2018 **Article Revised on 06/072018** **Article Accepted on 26/07/2018**

ABSTRACT
The presence of mercury metal in soil and water is negligible and there is hardly any literature available to establish microbial resistance to mercury toxicity. In the presence study four different strains were isolated from root rhizosphere region of *Vigna radiata* L. which were grown in soil collected from industrial area of Berhampur , Odisha. These four strains showed a wide range of resistance to mercury. A decrease in bacterial growth was observed with the increase in metal concentration at any given time interval compared to the control without metal amendment. The lower optical density values revealed that the bacterial growth was affected due to the presence of metal in the growth medium. The MRB (p2) thus isolated may be recommended their potential to be exploited in bioremediation purposes.

KEYWORDS: Heavy metal, Mercury, Soil bacteria, Rhizosphere.

INTRODUCTION

Microbial population responses to heavy metal contamination provide a relevant model for ecological studies to assess the influence of environmental characteristics (Guo et al., 2009). Several studies have demonstrated that metals influence microorganisms by affecting their growth, morphology and biochemical activity (Sandaa et al., 2001; Tsai et al., 2005; Pérez-de-Mora et al., 2006) and diversity (Dell‟Amico et al., 2008). The response of the bacterial populations to heavy metal contamination depends on the concentration and bioavailability of metals itself and is dependent by multiple factors such as the type of metal and microbial species (Hassen et al., 1998). High concentrations of metals (both essential and non-essential) harm the cells by displacing the enzyme metal ions, competing with structurally related non-metals in cell reactions and also blocking functional groups in the cell biomolecules (Hetzer et al., 2006). Microbial survival in heavy metal polluted soils depends on intrinsic biochemical properties, physiological and/or genetic adaptation including morphological, as well as environmental modifications of metal speciation (Abou-Shanab et al., 2007).

Studies on the effects of metals on soil bacteria have been conducted showing that short term contact causes the selection of resistant bacteria within weeks. A more prolonged exposure to metals slowly selects resistant bacteria. On the other hand long term exposure to metals leads to the selection/adaptation of the microbial community which then thrives in polluted soils (Pérez-de-Mora et al., 2006 and Chihching et al., 2008). The presence of different metals together may also have greater adverse effects on the soil microbial biomass/activity and diversity than those caused by single metals at high concentrations (Renella et al., 2005). Various microorganisms show a different response to toxic heavy metal ions that confer them with a range of metal tolerance (Valls and de Lorenzo, 2002). Bacteria may achieve this in different ways either through biological, physical or chemical mechanisms that include precipitation, complexation, adsorption, transport, product excretion, pigments, polysaccharides, enzymes, and specific metal binding proteins (Gadd, 1992; Marazioti, 1998; Hetzer et al., 2006).

As a response to toxic mercury compounds globally distributed by geological and anthropogenic activities, microbes have developed a surprising array of resistance mechanisms to overcome Hg toxicity (Pahan et al., 1990). However, some bacterial communities residing in the mercury contaminated areas can exchange mercury resistance genes between each other, because of continually exposure to the toxic levels of mercury.

Mercury, the only metal in liquid form at room temperature is the most toxic of the heavy metals and the sixth most toxic chemical in the list of hazardous compounds (White et al., 2005) has been present in the environment for aeons. Erupted from the core of earth by volcanic activity it exists as mineral (mostly as cinnabar-

HgS), as mercuric oxide, oxychloride, sulfate mineral (Kiyono and Pan Hau, 2006) or also as elemental mercury. Even small amounts of mercury are toxic for all organisms. Mercury binds to the sulfhydryl groups of enzymes and proteins, thereby inactivating vital cell functions (Dobler et al., 2000b). The most notable examples of environmental contamination with mercury occurred in Japan between 1953 and 1970 (Irukayama, 1966; Tsubaki, 1968). Pedersen and Sayler(1981) and Nordberg, (1976) found that $HgCl_2$ had no significant effects on methanogenesis. Microbial communities are constituted by structural clusters of microbial species, each playing different and complementary roles (Torsvik and Ovreas, 2002). The laboratory characteristics of an organism determined in vitro rarely reflect its real properties in the environment. Furthermore, several authors have confirmed that bacterial communities, diversity and structure are influenced by spatial and temporal variables such as temperature (Panswad et al., 2003), salinity (Bernhard et al., 2005), pH, nutrients present (Mills et al., 2003), and contamination with pollutants (Li et al., 2006). The environmental stress caused by heavy metals, generally decreases the diversity and activity of soil bacterial populations leading to a reduction of the total microbial biomass, decrease in numbers of specific populations such as rhizobia and a shift in microbial community structure (Sandaa et al., 1999; Wang et al., 2010).

The sediment environment may protect the methanogenic population from the toxic effects of mercury (Pederson and Sayler, 1981). Many bacteria possess a variety of resistance mechanisms to the toxic effects of mercury. Resistance depends on the strain, species, and genus of bacteria. Nelson and Colwell(1975) showed that H_2S production is not an exclusive property of mercury resistant bacteria. Microorganisms are capable for chemical reduction and removal of mercury salts from waste water. Microorganism activities contribute to the biological cycle of mercury in the environment. Some bacteria are capable to transform mercury into harmless form showed a positive correlation between the presences of resistant microorganism with the distribution of mercury compounds in contaminated sediments. The detoxification mechanism of mercury by microorganism may be represented with methylation process which is conducted by bacteria (Robinson and Touvinen, 1984).

MATERIALS AND METHODS

Sample Collection /Sampling: Soil samples were collected from different sites of Berhampur, Odisha and kept in different pots for planting of selected seeds.

Selection of Seeds: Seeds of mung bean (*Vigna radiate* L. OBGG-52 Durga) are collected from Krishi vigyan Kendra, Ratnapur, Ganjam.

Germination of Seeds and Preparing Samples: The soil sample from rhizosphere region of plant was collected for 10 fold serial dilution technique.

Enumeration of Viable Cells: 10 fold serial dilution technique was carried out. Aliquotes of 0.1ml for each dilution as spread on Nutrient Agar media plates. The plates were incubated at 37°C /24 hours. After 24 hours colonies are observed.

Isolation of Bacteria

Nutrient agar media was prepared and poured in the petriplates. After solidifying 0.1ml of soil sample from each dilution was spread on each plates of different dilution were kept in incubator for 24 hours /37°C. After 24 hours the growth of bacterial colonies were observed. From spread plate the bacteria inoculated to streak plate for isolation of bacterial colonies.

Colony Morphology

Size, shape, colour of the bacterial colonies were observed after 24 hours incubated cultures, on the Nutrient agar (NA). Pure colonies were isolated and kept in slants at 4°.

Determination of the Effect of Metals on Bacterial Growth

The lowest concentration of metal that completely prevented bacterial growth (Gupta et al., 2005). Tolerance of isolates was done by tube dilution method. Different concentration of mercury chloride solution was prepared ranging from 1ppm, 5ppm, 10ppm, 15ppm, 20ppm concentrations. Nutrient broth amended with the heavy metal and inoculated exponentially growing culture (24 hour old, OD of 0.090 at 600nm) of bacterial isolates prepared in the same medium. Medium with metal but without bacteria is taken as control. All the experiments were conducted in triplicate. All the test tubes were incubated at 37°C for 24 hours. Bacterial grow was measured in terms of optical density at 600nm. Growth curves of bacteria isolates were plotted.

Antimicrobial Susceptibility Testing

The isolate (p2) that presented tolerance to mercury was tested for susceptibility to antimicrobial agents by disc diffusion method (Kirby and Baur et al; 1996). The antimicrobial agents used were streptomycin (10mg per disc), gentamicin (10mg per disc), norfloxacin (10mg per disc), ciprofloxacin (5mg per disc).

RESULTS

Physicochemical Properties of Soil Sample

Soil samples were collected from different sites of Berhampur, was determined for physicochemical properties as Shown in Table -1. It was found that it as neutral soil (pH 6.9) with a relatively high content of potash, organic carbon and low content of available potash.

Table 1: Physiochemical properties.

Soil properties	Values	Nature
pH	6.9	Neutral
Electrical conductivity	0.62	Normal
Available phosphorus	84.33	High
Organic carbon	1.8	High
Available potash	84.33	Low

Colony Morphology

The observed colony morphological characteristics pretending to colour, shape and elevation are collectively displayed on table-2.

Table 2: Morphological characteristics.

Bacterial isolates	Colour	Shape	Elevation
p1	Whitish	Rhizoid	Flat
p2	Pale yellow	Irregular	Raised
p3	Whitish cream	Rounded	Flat
p4	Whitish cream	Irregular	Convex

Cell Morphology

Cell morphology of isolate was studied and observation are described as in table-3.

Table 3: Cell morphology of isolated strains.

Bacterial strain	Colour	Gram staining	Shape
p1	Purple	+ve	Bacillus
p2	Purple	+ve	Bacillus
p3	Purple	+ve	Bacillus
p4	Pink	-ve	Cocci

Determination of the Effect of Metals on Bacterial Growth

The metal response experiments were carried out in a nutrient broth supplemented with different concentration of mercury chloride solution. Different isolates exhibited different growth patterns in the presence of different concentration of heavy metal.

Table 4: Absorbance values of isolates (p1,p2,p3,p4) at different ppm concentration.

		bacteria isolates		
ppm (parts per million) concentration	p1	p2	p3	p4
1ppm	0.2	0.35	0.15	0.35
5ppm	0.17	0.26	0.08	0.17
10ppm	0.08	0.19	0.03	0.06
15ppm	0.02	0.11	0.01	0.03
20ppm	0	0.025	0	0

All the isolates exhibited better growth upto 10ppm concentration and further their growth gradually decreases at 15ppm concentration. Only one isolate (p2) is able to tolerate upto 20ppm concentration while others were inhibited. A decrease in growth measured in terms of optical density at 600nm was observed with increasing metal concentration at any given time interval. The lower optical density values revealed that the bacterial growth was affected due to the presence of metal in growth medium. However it is not easy to make a meaningful comparision with the finding reported in the literature.

Antibiotic Sensitivity Test

Antibiotic sensitivity test was carried out by (Baur et al; 1996). It was found that isolate p2 showed resistance to norfloxacin (10mg per disc) while sensitive to other antibiotics taken in experiment.

Table 5: Isolate (p2) response to antibiotic.

Antibiotics	Resistant
Streptomycin (10mg per disc)	-ve
Gentamicin (10mg per disc)	-ve
Norfloxacin (10mg per disc)	+ve
Ciprofloxacin (5mg per disc)	-ve

DISCUSSION

In the present study nutrient agar is used for isolation of soil bacteria from contaminated sites. Mercury resistant bacteria were isolated by tube dilution technique using nutrient broth containing mercury of different concentration. A total of four bacteria were isolated resistant to different mercury concentrations in ppm (parts per minute). Heavy metals are known to alter the functional diversity of soil, microbial community and impair specific pathways of nutrient cycling (Ramteke et al., 2012). It is known that heavy metal pollution causes selection and / or development of tolerant microorganisms.

The organic content in soil samples is considered as one of the key determinants driving the microbial community structure (Roane and Kellogg, 1996; Zhou et al., 2002). Soil with high heavy metal content also had a high organic content, which can probably explain the maintenance of the microbial community diversity due to lack of competition, as suggested by others authors (Zhou et al., 2002; Branco et al., 2005; Ramteke et al., 2012). Microorganisms have developed the mechanisms to cope with a variety of toxic metals for their survival in the environment enriched with such metals (Martin – Laurent et al., 2004). Heavy metals exert toxic effects on the microorganisms through various mechanisms and metal tolerant bacteria could be isolated and selected for their potential application in the bioremediation of contaminated soil. Heavy metals resistant microorganisms which grow not only under contaminated environment but also possess growth promoting properties are of particular importance for the degraded and polluted land use practice (Joseph et al., 2007; Ramteke et al., 2012).

Factors such as the culture media employed, growth conditions, and incubation period. Besides the various possible forms and concentrations of metals used in the tests of tolerance, May be difficult for standardization and influence the in-vitro toxicity of the metals. Due to these facts there are no universally accepted metal concentrations to define bacterial tolerance or resistance. The percentage of mercury tolerant strains from sources were compared to the different metal concentration tested, especially samples from the garbage sewage. Considering that tolerance to mercury is probably due to mercury genes which are often associated with genes that confer resistance to antimicrobial drugs.

ACKNOWLEDGEMENTS

The authors are thankful to the Vice Chancellor of Khallikote University and Principal of Khallikote Autonomous College, Berhampur, Odisha for encouragement of research activity and providing necessary laboratory facilities.

REFERENCE

1. Abou-Shanab, R.A.I., Van Berkum, P. & Angle, J.S.: Heavy metal resistance and genotypic analysis of metal resistance genes in gram-positive and gram-negative bacteria present in Ni-rich serpentine soil and in the rhizosphere of *Alyssum murale*. Chemosphere., 2007; 68: 360-367.
2. Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M: Antibiotic susceptibility testing by a Standardized single disc method. Amer. J. Clin. Pathol., 1996; 45: 493-496.
3. Bernhard, A.E., Colbert, D., McManus, J. & Field, K.G: Microbial community dynamics based on 16S rRNA gene profiles in a Pacific Northwest estuary and its tributaries. FEMS Microbiology Ecology., 2005; 52: 115-128.
4. Branco, R., Chung, A., verssimo, A. and Morais, P.V.,: Impact of Chromium contaminated waste waters on the microbial community of a river. FEMS Microbiol. Ecol., 2005; 54: 35-46.
5. Chihching, C., Yumei, K., Changchieh, C., Chunwei, H., Chihwei, Y. & Chunwei, Y.: Microbial diversity of soil bacteria in agricultural field contaminated with heavy metals. Journal of Environmental Sciences., 2008; 20: 359-363.
6. Dell'Amico, E., Mazzocchi, M., Cavalca, L., Allievi, L. & Andreoni, V.: Assessment of bacterial community structure in a long-term copper-polluted ex vineyard soil. Microbiological Research., 2008; 163: 671-683.
7. Dobler Wagner, I., Von Caustein, H., Timmis, K.N., Li, Y. and Deckwer, W.D.,: Removal of mercury from chemical wastewater by microorganisms scale. Environ. Sci. Technol., 2000b; 34: 4628-4634.
8. Gadd, G.M.: Microbial control of heavy metal pollution. Microbial control of pollution, 48th Symposium of the Society for General Microbiology, Cambridge University Press, 1992; 59-88.
9. Guo, Z., Megharaj, M., Beer, M., Ming, H., Rahman, M. M., Wu, W. & Naidu, R.: Heavy metal impact on bacterial biomass based on DNA analysis and uptake by wild plants in the abandoned copper mine soils. Bioresource Technology., 2009; 100: 3831-3836.
10. Gupta, A., Rai, V., Bagdwal, N., Goel, R.: In situ characterization of mercury – resistant growth promoting florescent pseudomonas. Microbiological Research., 2005; 160: 385-388.
11. Hassen, A., Saidi, N., Cherif, M. & Boudabous, A.: Resistance of environmental bacteria to heavy metals. Bioresource Technology., 1998; 64: 7-15.
12. Hetzer, A., Daughney, C.J. & Morgan, H.W.: Cadmium Ion Biosorption by the Thermophilic Bacteria Geobacillus stearothermophilus and G. thermocatenulatus. Applied and Environmental Microbiology., 2006; 72: 4020-4027.
13. Irukayama: The pollution of Minamata Bay and Minamata Disease. J. Water Poll. Control Fed., 1966; 38: 384-385.

14. Joseph, B., Ranjan, Lawrence, R: characterization of plant growth promoting rhizobacteria associated with chickpea. Int. J. of plant production., 2007; 2: 141-152.
15. Kiyono, M. and PanHau, H: Genetic engineering of bacteria for environmental remediation of mercury. J. Health Sci., 2006; 52: 199-204.
16. Li, Z., Xu, J., Tang, C., Wu, J., Muhammad, A. & Wang, H.: Application of 16S rDNA-PCR amplification and DGGE fingerprinting for detection of shift in microbial community diversity in Cu, Zn, and Cd contaminated paddy soils. Chemosphere., 2006; 62: 1374-1380.
17. Marazioti, C.: Heavy Metal Tolerance and Uptake by Soil Bacteria. Institute of BioScience and Technology: Cranfield University, 1998.
18. Martin Laurent, F., Ranjard, L., lopez, J. Gutierrezand L.philppot : Estimation of atrazine degrading genetic potential and activity in three French agricultural soil. FEMS microbial. Ecol., 2004; 48: 425-435.
19. Nelson, J.D., Jr. and Colwell, R.R., (1975) The ecology of mercury resistant bacteria in Chesapeake Bay. Microbial Ecol., 1975; 1: 191-218.
20. Panswad, T., Doungchai, A. & Anotai, J: Temperature effect on the microbial community of enhanced biological phosphorus removal system. Water Research, 2003; 37: 409-415.
21. Pedersen, D. and Sayler, G.S.: Methanogenesis in freshwater sediments: inherent variability and effects of environmental for bacterial methylation of mercury. Proc. Lan. Aquatic Tox. Workshop, (1981).
22. Pérez-de-Mora, A., Burgos, P., Madejón, E., Cabrera, F., Jaeckel, P. & Schloter, M.(2006) Microbial community structure and function in a soil contaminated by heavy metals: effects of plant growth and different amendments. Soil Biology & Biochemistry., 2006; 38: 327-341.
23. Ramteke, P.W., Joseph, B., Mani, A. and Chacko, S.(2012) *Pisum sativum* and associated plant growth promoting rhizobacteria :Effect of normal and sewage irrigation. Int. J. of soil sci., 2012; 1816-4978.
24. Renella, G., Mench, M., Gelsomino, A., Landi, L. & Nannipieri, P.: Functional activity and microbial community structure in soils amended with bimetallic sludges. Soil Biology & Biochemistry., 2005; 37: 1498-1506.
25. Roane, T.M. and Kellog, S.T.: characterization of bacterial communities in heavy metal contaminated soils .Can j. Microbial, 1996; 42: 593-603.
26. Robinson, J.B. and Touvinen, O.H.: Mechanism of microbial resistance anddetoxification of mercury and organomercury compounds: Physiological, biochemical and genetic analysis. Microbiological Review., June 1984; 95-124.
27. Sandaa, R.A., Torsvik, V., Enger, Ø., Daae, F.L., Castberg, T. & Hahn, D. :Analysis of bacterial communities in heavy metal-contaminated soils at different levels of resolution. FEMS Microbiology Ecology., 1999; 30: 237-251.
28. Sandaa, R.A., Torsvik, V. & Enger, Ø.: Influence of long-term heavy-metal contamination on microbial communities in soil. Soil Biology & Biochemistry., 2001; 33: 287-295.
29. Torsvik, V. & Ovreas, L.: Microbial diversity and function in soil: from genes to ecosystems. Current Opinion in Microbiology., 2002; 5: 240-245.
30. Tsai, Y.P., You, S.J., Pai, T.Y. & Chen, K.W.: Effect of cadmium on composition and diversity of bacterial communities in activated sludges. International Biodeterioration & Biodegradation., 2005; 55: 285-291.
31. Valls, M. & de Lorenzo, V.: Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution. FEMS Microbiology Reviews., 2002; 26: 327-338.
32. White, D.G., Alkeshun, M.N. and Mc Dermott, P.F.: Frontiers in Antimicrobial Resistance, Chapter 11, Metal Resistance Loci of Bacterial Plasmid. 1st Edn. ASM Publication, USA., 2005; 165-173.
33. Wang, F., Yao, J., Si, Y., Chen, H., Russel, M., Chen, K., Qian, Y., Zaray, G. & Bramanti, E.:Short-time effect of heavy metals upon microbial community activity. Journal of Hazardous Mate rials., 2010; 173: 510-516.
34. Zhou, J., Xia, B., Treves, D.s., Wu, L.Y., Marsh, T.L., O'Neill, R.v., Palumbo, A.V., Tiedje, J.M.: Spatial and resource factors influencing high microbial diversity in soil. Appl. Environ. Microbiol., 2002; 68: 326-334.