Journal of Chemical Health Risks 1(1): 19-22, 2011

Separation of Mercury Resistant Bacteria from Wastewater of Milk, Detergent and Ceramic Industry

M. Moghbeli [1], F. Shakeri [1] and H. Hashemi-Moghaddam [2]*

[1]Department of Microbiology, Damghan Branch, Islamic Azad University, P.O. Box 36716-39998, Damghan, Iran
[2]Department of Chemistry, Damghan Branch, Islamic Azad University, P.O. Box 36716-39998, Damghan, Iran

Abstract: Use of microorganisms for removing mercury is an effective technology for the treatment of industrial wastewaters and can become an effective tool for the remediation of man-impacted coastal ecosystems with this metal. In this study, seven types of mercury resistant bacteria were separated from industrial waste and minimum inhibitory concentration (MIC), were determined for these bacteria. Results showed that two strains of bacteria, which isolated from waste water detergent plants, are more resistant to mercury and able to grow at the presence of 52 ppm of mercuric chloride. These bacteria could be used for biological treatment of mercury in contaminated wastewater.

Keywords: Biosorption; Bacteria; Mercury sorption, Industrial waste

INTRODUCTION

Mercury, 6th most abundant toxic element is released into the environment as a result of both natural processes as well as due to anthropogenic activities (Devars *et al*, 2010; Arif Tasleem *et al*, 2009). In nature, mercury exists in three forms: Metallic or elemental form (Hg^0), inorganic (Hg^{2+} or Hg^+) and organic form ($R\text{-}Hg^+$ or R-Hg-X, where ''R'' is methyl or phenyl and ''X'' is acetate) (Clarkson *et al*, 2003; Poulain *et al*, 2007).organic form being the most toxic and elemental as the least one. Toxicity to organic mercury is accredited due of its lipid solubility, which it employs while crossing the placental and blood–brain barrier (Clarkson *c* 2003; Poulain *et al*, 2007; Summers, 2009). In spite of the fact that both organic as well as inorganic forms of mercury crosses the membrane with different abilities; both have high affinity for thiol groups of enzymes and proteins. Results in inhibition of activity of a wide variety of enzymes, interferes with membrane transporter proteins and disturbs structural integrity of cell by disturbing microtubule formation (Summers, 2009; Mortazavi *et al*, 2005). Longer exposure to mercury also results in the inhibition of RNA and protein synthesis that ultimately leads to retardation of cell growth (Mortazavi *et al*, 2005). Mercury is a neurotoxic metal having diverse effects on the cellular functions of the brain.

Organic mercury crosses blood brain barrier and as such accumulates in the motor regions of the brain and central nervous system (CNS), where it damages nerve cells and generates high level of reactive oxygen species(Poulain *et al*, 2007; Adebowale Adeniji, 2004; Okino *et al*, 2000).

Due to persistence of various forms of mercury in natural environments, large number of microorganisms in particular bacteria have developed resistance to mercury and as such are playing major role in environmental decontamination of mercury, and the use of microorganisms in the removal of metals from contaminated wastewater, mining, and industrial wastes is generally considered promising (Essa *et al*, 2005; Rasmussen *et al*, 2008; Ruiz & Daniell 2009). Mercury-resistance determinants are found in a wide variety of both Gram negative as well as Gram-positive bacteria. Bacteria have developed a surprising array of resistance mechanisms based on clustering genes in a single

Corresponding Author: Moghbeli M, Department of Microbiology, Damghan Branch, Islamic Azad University, P.O. Box 36716-39998, Damghan, Iran

operon referred as ‘‘mer operon’’. Genes encoded by the mer operon have been reported to be located on plasmid, chromosomes, transposons as well as on integrons (Schue *et al* , 2009; Moshafi *et al*, 2009; Jaysankar De *et al*, 2003). In this study, mercury resistant bacteria were separated from waste water of milk and ceramic industry and the effects of various mercury concentrations were investigated.

METHODS

Sample Collection

50 ml samples were collected from the waste water of detergent, milk and ceramic plants in glass sterile containers and were transferred to the laboratory in the presence of ice at maximum four hours.

Isolation and identification of mercury resistant bacteria

Separation of mercury resistant bacterium was performed by primary enrichment method.
In primary enrichments, first one milliliter of waste sample was added to 9 ml of LB Broth medium which contains 20 ppm of mercury. Mediums were incubated at 30 ^{0}C for 48 hours.
Then 100 µL of these samples were cultured on LB Agar medium containing of the same amount of mercury and again, incubated at 30 ° C. Pure samples were prepared from formed colonies by streak culture method after 24 hours,
The initial identification of the purified bacteria was performed by gram staining, catalase and oxidase tests.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was known as standard for determining susceptibility of microorganisms to antimicrobial materials. So it is preferable to other tests. In this study, to determination of MIC, a colony of examined bacteria from Medium of LB agar was inoculated into 5 ml of the LB broth and incubated within 18 to 24 hours at 37 °C until produce the one McFarland turbidity. Stock 20 ppt solution of mercury was prepared by dissolving of 100mg of mercury in 5 ml of sterile water. For determination of MIC for each bacterium, to ten tubes containing of 5 ml of LB broth following amounts of mercury chloride was added and then 100 µL from each bacterium with one McFarland turbidity was added and incubated within 24 hours at 37 °C.

Table 1. Concentration of mercury in tubes containing of 5 ml of LB broth

No.	Amount of added Hg(Cl)2 (µL)	Concentration of $Hg(Cl)_2$ ppm
1	2.5	10
2	5	20
3	7	28
4	9	36
5	11	44
6	13	52
7	15	60
8	17	68
9	19	76
10	21	84

Concentration of mercury chloride was considered as MIC in the first tube which bacteria do not grow. Concentration of mercury chloride in the tube before of MIC is amounted of mercury which the bacteria are resistant to it.

RESULTS AND DISCUSSION

In this study, four waste samples were collected from milk and ceramic industry, and samples were cultured on medium containing mercury.

Bacteria isolated from the wastewaters showed different degrees of resistance to mercuric chloride.

So that, the greatest and least resistances were for bacteria isolated from the detergents and milk factory respectively. Two gram-negative rod bacteria and gram-positive cocci bacteria were isolated from waste of ceramic factories. Which MIC of both was 5 and are resistance to concentration of 36 ppm of mercury. Furthermore, two gram-negative and gram-positive rod bacteria were isolated from waste of first milk factory. Which MIC of both was 2 and are resistance to concentration of 10 ppm of mercury.

From the waste of second milk factory, a gram-positive cocci bacteria was isolated which MIC of it was 2 and is resistance to concentration of 28 ppm of mercury.

Finally, in the waste of detergent factory, two kinds of gram –negative rod bacteria were isolated, which have different colony, catalase and oxidase properties. MIC of both was 7 and is resistance to concentration of 52 ppm of mercury. This is the greatest observed resistance.

Table **2**. Kinds of Bacteria isolated from the different waste water, MIC and Mercury resistance concentration (ppm)

Kind of Wastewater	Bacteria	MIC	Mercury resistance Concentration(ppm)
Ceramic	Gram negative bacilli and gram positive cocci	5	36
Milk 1	Gram positive and gram negative bacilli	2	10
Milk2	Gram positive cocci	4	28
Detergent	Gram negative bacilli	7	52

CONCLUSION

Increased levels of mercury in natural resources from human activities cause the resistance of microorganisms to heavy metals. By isolation of these microorganisms, those can be used for the bioremediation of waste water (Devars *et al*, 2010; Arif Tasleem *et al*, 2009; Clarkson *et al*, 2003; Poulain *et al*, 2007; Summers, 2009).

Variety of microorganisms which are resistant to mercury, especially bacteria, were isolated from wastewater, hot water, seawater and soil and used for biological detoxification.(Adebowale Adeniji ,2004; Okino , 2000; Moshafi , 2009; Jaysankar De , 2003) In this study for the first time two bacteria were isolated with high resistance to mercury from waste of detergent factory. Waste samples were cultured on medium containing mercuric chloride and formed colonies purified and the bacterial were investigated.

Gram-negative bacteria are more resistant to mercury rather than gram-positive bacteria. Two yellow and cream-colored gram-negative, colonies were more resistant to mercury, which was 52 ppm.

REFERENCES

Adebowale A., 2004. Bioremediation of Arsenic, Chromium,Lead, and Mercury, National Network of Environmental Management Studies Fellowfor U.S. Environmental Protection Agency. www.clu-in.org

Arif Tasleem J., M. Imtiyaz, A. Arif and H. Rizwanul, 2009. Mercury pollution: an emerging problem and potential Bacterial remediation strategies., World J Microbiol Biotechnol 25:1529–1537

Clarkson T. W., L. Magos and G. J. Myers, 2003. Toxicology of mercury;current exposure and clinical manifestations. N Engl J Med 349:1731–1737

Devars S., J. S. Rodríguez-Zavala and R. Moreno-Sánchez, 2010. Enhanced Tolerance to Mercury in a Streptomycin-Resistant Strain of *Euglena gracilis*, Water, Air, & Soil Pollution

Essa A. M. , L. E. Macaskie and N. L. Brown, 2005. A new method for mercury removal, Biotechnology Letters (2005) 27: 1649–1655

Jaysankar De , N. Ramaiah, A. Mesquita and X. N. Verlekar. (2003), Tolerance to Various Toxicants by Marine Bacteria Highly Resistant to Mercury., Marine Biotechnology

Mortazavi S., A. Rezaee, A. Khavanin, S. Varmazyar and M. Iafarzadeh ,2005. Removal of Mercuric Chloride by a Mercury Resistant Pseudomonas putida Strain. Journal ofBiological Sciences 5 (3): 269-273

Moshafi M.H., Mansori S., Nemati R., Forootanfar H.(2009), Simultaneous Resistance to heavy Metals and Antibiotics in Escherichia coli Strains Isolated from Clinical Samples. Rafsanjan Med. Sci. Univer. J. , (8), 1193-202

Okino S., K. Iwasaki, O. Yagi and H. Tanaka, 2000. Development of a biological mercury removal-recovery system., Biotechnology Letters **22:** 783–788

Poulain A. J., S. M. N. Chadhain, A. P. Ariya, M. Amyot, E. Garcia, P. G. C. Campbell, G. J. Zylstra and T. Barkay, 2007. Potential for mercury reduction by microbes in high Arctic. Appl Environ Microbiol 73(7):2230–2238

Rasmussen LD, Zawadsky C, Binnerup SJ, Oregaard G, Sorensen SJ,Kroer N (2008) Cultivation of hard to culture subsurface mercury resistant bacteria and discovery of new merA gene sequences. Appl Environ Microbiol 74(12):3795–3803

Ruiz NO, Daniell H (2009) Genetic engineering to enhance mercury phytoremediation. Curr Opin Biotechnol 20:1–7

Schue M, Dover LG, Besra GS, Parkhill J, Brown NL (2009) Sequence and analysis of a plasmid encoded mercury resistance operon from Mycobacterium marinum identifies MerH, a new mercuric ion transporter. J Bacteriol 191(1):439–444. Doi

Summers A. O., 2009. Damage control: regulating defenses against toxic metals and metalloids. Curr Opin Microbiol 12:1–7