Punjab Univ. J. Zool. Vol. 12, pp. 107-113, 1997

PREDOMINANCE OF LEAD RESISTANT AND LEAD PROCESSING BACTERIA IN INDUSTRIAL AND SEWAGE WASTES

RIAZ UL HAQ, ADIL MAHMOOD, ABDUL REHMAN, SYED SHAHID ALI AND ABDUL RAUF SHAKOORI[1]

Cell and Molecular Biology Laboratory, Department of Zoology, University of the Punjab, Quaid-i-Azam Campus, Lahore, 54590, Pakistan

Abstract: Twenty six samples from industrial wastes and sewage waste were collected and checked for the presence of lead resistance bacteria. Nineteen lead resistant bacterial strains were isolated. Most of the bacteria showed resistance against lead up to a concentration of 1 mg/ml. of the medium. Seven bacterial strains were selected to determine their lead processing ability. The bacteria showed efficient capability to process lead. The mechanism for lead processing is proposed to be lead accumulation or lead sorption. Variation in the lead processing ability of the bacteria was observed. Most of the isolates showed an initial speedy processing of lead during 24 hours and a slower relatively constant processing during next 48 hours. One of the isolates showed a slow processing during first 24 hours and a more enhanced processing during the next 24 hours and a very speedy processing during the next 24 hours. Plasmids were detected in some of the strains capable of lead processing showing that the gene for lead processing was usually present on chromosomal DNA. Plasmid occurrence frequency was more in an area where there was constant inpouring of industrial wastes for the last many (~50) years while it was less in other areas where there were separate ponds.

Key words: Biodegradation, heavy metals, lead resistance, lead accumulation, industrial pollution.

INTRODUCTION

Lead resistant bacteria are frequently found in sewage water and industrial wastes containing lead. A number of scientists have reported isolation of lead resistant bacteria (Couillard and Chartier, 1993; Tondwalkar *et al.*, 1990; Bender *et al.*, 1989; Lee *at al.*, 1992). Most of the developing countries like Pakistan are facing the problem of heavy metal contamination through industrial wastes while these wastes are not being treated properly. With increasing insight into the capability of a variety of microorganisms to process and detoxify heavy metal ions, the scientists have suggested to exploit this capability in environmental clean up operations. Bioremediation or the use of microorganisms for decontamination is getting the status of first rate method for environmental protection. Microorganisms used for these

0079-8045/97/0001-0101 $ 03.00/0

operations not only detoxify heavy metal ion in the environment but also don't add any surplus chemicals as is usually the result of chemical treatment plants. Metal processing strains have now been commonly reported in a number of studies. These strains have been studied and improved for exploitation in detoxification procedures. Lead resistant bacteria have also been used for lead and other metal ion processing. In a study bacterial strains were used to adsorb metals like lead, copper and zinc from spent wash. One of the strains removed 64% lead in one hour and 82% copper in two hours, while the other strain removed 76% of the zinc in two hours (Tondwalkar *et al.*, 1990). Another study reports the use of a technique of integrated ecosystem for the uptake of lead. The transfer of metal through the system was observed with the eventual binding of the metal with the biomass. It was noted that increase in biomass of microorganisms increased lead-recovery. It also increased microbial tolerance for lead. The stable microbial mass floating on the surface of the pond bound the metal for extended period of time (Bender *et al.*, 1989).

The aim of this study was to check the presence of lead resistant and lead processing bacteria in various sewage wastes and industrial effluents. In this regard nineteen bacterial isolates from water samples got from various industrial waste were checked for their lead resistance. Seventeen bacterial isolates showed resistance against high concentrations of lead in the medium. Lead processing ability of the isolates was checked by estimating the concentration of lead in the medium after various intervals of growth of bacteria in the cultures. The reduction in the amount of lead indicated the processing of lead by bacterial strains. Two modes of processing of lead were observed. The implications of the lead resistant bacteria in bioremediation and environmental cleanup operations were discussed.

MATERIALS AND METHODS

Collection of samples

Twelve water samples were collected from effluents released by tanning industry, 4 from effluents of industry engaged in food processing, soap formation and textile mixed with sewage waste and three from effluents of ICI paint plant. The samples were collected in sterile screw capped glass bottles, brought to the laboratory and stored at room temperature before spreading on plates.

Growth and lead resistance of bacteria

For selection of lead resistance bacteria LB agar plates with 1mg/mL lead were used. Lead acetate was used as source of Pb^{2+}. Lead acetate solution and LB agar medium were autoclaved separately and allowed to cool down. When the temperature of the two solutions was slightly less than 60°C, the solutions were mixed and poured into

plates. Industrial effluent (100µl) was spread on the plates and the growth of colonies was observed after 24 hours. Colonies were picked and streaked for purification and for determination of maximum resistance of the strains against lead.

Estimation of lead in the medium

In order to estimate the amount of lead in the medium, dithizone method (E. Merck) was used. Reagent I (R1) was prepared by dissolving 10mL of hydrazinium hydroxide in 70mL of 1N HCl, then adding 20g of NaCl and making the volume up to 100mL with distilled water. Reagent 2 (R2) was prepared by dissolving 20g potassium hydrogen carbonate, 5g potassium cyanide, 5g potassium sodium tartarate and 25mL ammonium solution in 100mL of distilled water. Dithizone solution was prepared by dissolving 15mg of dithizone in 1000mL of chloroform. Five mL of LB broth with lead concentration of 1mg/mL was taken in test tubes. The tubes were inoculated with fresh bacterial cultures. After incubation for 24 hours 1mL culture was taken from the tube aseptically. The culture was diluted to 25 times volume with distilled water. This mixture was taken in a separating funnel and 2.5mL of R1 were added followed by addition of 2.5mL of R2. After that 12.5mL of dithizone solution was added. The mixture was shaken for 5 minutes and the pressure developed by shaking and reaction was released by removing the stopper or valve. The mixture was allowed to stand for a few minutes. Two layers were formed. The lower layer containing chloroform was collected in a glass bottle and OD of the solution was taken at 515nm against a blank which was prepared through a similar procedure by taking 25mL distilled water without any culture. The same procedure was adopted to estimate lead at 0, 48, and 72 hours. Control of the lead processing was run using the medium without inoculation incubated at same conditions as that of the culture.

Amount of lead was calculated by the following formula:

$$G = \frac{M \times 95.4 \times \text{dilution factor}}{a}$$

where G = amount of lead in mg/L, a = amount of water used in mL, M = absorbance. Bacterial processing of lead was assessed by estimating the amount of lead in the medium after various time intervals. All the readings were taken in triplicate for statistical analysis.

Isolation of plasmids

A single colony of various lead resistant strains was selected and grown in LB liquid medium for plasmid isolation. The procedure adopted for isolation of plasmids was as described by Holmes (1984). Plasmid DNA isolated from the bacterial isolates

was run on agarose gel and visualized under UV illuminator after staining with ethidium bromide. The isolation experiment was repeated three times to ascertain the presence of the plasmids.

RESULTS

Isolation of lead resistant bacteria

LB agar plates containing lead at a concentration of 1mg/mL were used to isolate lead resistant bacterial isolates. Seventeen isolates showed resistance against lead concentration of 1mg/mL while two isolates showed resistance against lead concentration of 2mg/mL of the medium. Single colonies were picked and inoculated in 250mL flasks containing 100 mL of the LB liquid medium. The pH of the medium was adjusted at 7.0 and the temperature of incubation was 30°C.

Processing of lead by bacterial isolates

Processing of lead by the bacterial strains was determined though dithizone method. Five strains were used for checking lead processing efficiency. The results are shown in Table I and Fig. 1.

Table I: Percentage reduction after various time intervals in amount of lead present in the medium inoculated with fresh cultures of bacterial isolates

	Time (Hours)		
Isolate No.	24	48	72
	Lead Reduction (%)		
	66.33*	67.43	68.43
2	64.23	64.79	65.12
3	69.53	76.37	76.93
4	2.64	30.0	79.0
7	50.0	52.64	55.29

*All the values in the table are mean of three readings

Isolation of plasmids

Twelve isolates from the effluents of Kasur tanneries waste were used for plasmid isolation and nine (75%) out of these were found to harbor plasmids. Three isolates from ICI paint plant wastes were used for plasmid isolation and none of these was found to harbor a plasmid. Four isolates from effluents of Kot Lakhpat industrial area of Lahore were used for isolation of plasmids and only one strain (25%) was found to contain a plasmid.

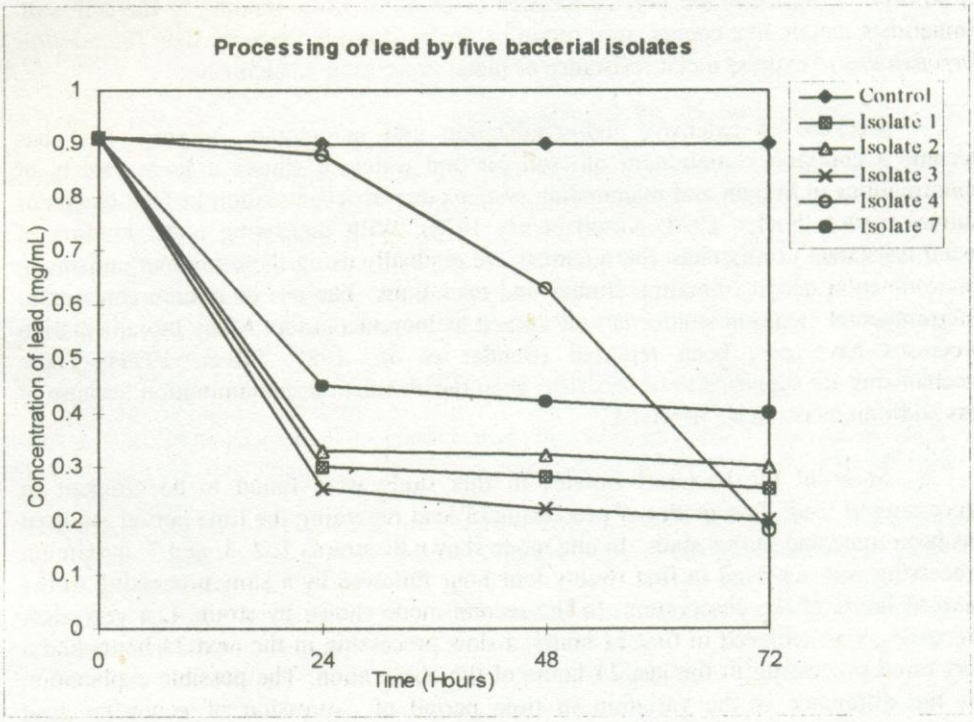

Fig. 1: Graph showing the amount of lead in the media estimated at various time intervals after inoculation of the media with exponential phase cultures of various bacterial isolates.

DISCUSSION

Metal tolerant microbial strains have been frequently reported in water samples taken from the environment receiving metal ions. They originate in habitats having elevated levels of heavy metals (Verma, 1995). Bacterial resistance mechanisms have

extensively been studied and it is found that bacteria resist metals due to the presence of some cellular mechanisms of combating toxic effects of metals (Mergeay, 1991; Nies and Silver, 1995; Wood and Wang, 1985). Various metal resistance and metal processing mechanisms are involved in bacterial metal resistance. Generally these are reduction of metal and detoxification through the enzymes present at the cell wall or periplasmic space. In this process the reduced metal ions remain outside the cell membrane and usually in the medium. Another method of metal resistance is metal accumulation by the bacterial cells through ion influx and effluxes. In this process the metal ions sometimes accumulate inside the cell and may cause ultimate death of the cell. Still other method is biosorption or binding at the surface of the cell. In this process of adsorption the metal ion may be reduced or oxidized while binding to the cell wall. Sometimes metals like copper, iron or sulfur are oxidized by bacteria like *Thiobacillus ferrooxidans* to express metal resistance or metal processing mechanisms.

Because of extensive industrialization and automobile running, lead has become a common contaminant of soil air and water. It causes a large variety of abnormalities in human and mammalian systems due to accumulation in food chains or animal tissues (Sachs, 1978; Kusell *et al.*, 1978). With increasing understanding of metal resistance in microbes, the scientists are gradually using these microorganisms in environmental decontamination studies and operations. The use of microorganisms in environmental clean-up studies is now coined as bioremediation. Many bioremediation processes have now been reported (Bender *et al.*, 1989; Silver, 1994). These mechanisms are supposed to be less risky than the chemical decontamination because of less additional chemicals involved.

Some of the bacterial isolates in this study were found to be efficient in processing of lead. Two modes of processing of lead regarding the time period required has been indicated in this study. In one mode shown by strains 1, 2, 3, and 7, maximum processing was achieved in first twenty four hour followed by a slow processing in the next 48 hours of the observation. In the second mode shown by strain 4, a very slow processing was achieved in first 24 hours, a slow processing in the next 24 hours and a very rapid processing in the last 24 hours of the observation. The possible explanation for the difference is the variation in time period of expression of genes for lead processing. Secondly, some of the bacteria exposed to constant stress of lead may have developed an efficient system of lead processing which comes in operation quickly when exposed to lead while others may have to undergo a long genetic process for expression of lead processing proteins. Another conclusion which can be drawn from the results is that resistance to lead and processing of lead may be achieved through two different mechanisms.

Most of the strains got from tannery effluents were found to harbor plasmids which indicate that plasmid transfer took place in an area due to common environment

and contact of bacterial strains with one another. Secondly, the absence of plasmids in lead resistant strains also indicated that gene for lead resistance is not solely present on plasmids. It is present on the chromosome. The absence of plasmids in bacterial isolates from other effluents like that of ICI showed that the bacteria in the area had less possibility of encounter with plasmid bearing bacteria or the proper stress was not present for maintenance of plasmids.

REFERENCES

BENDER, J.A., ARCHIBOLD, E.R., IBEANUSI, V. AND GOULD, J.P., 1989. Lead removal from contaminated waters by a mixed microbial ecosystem. In: *Water pollution research and control* (ed. L. Lijklema), vol., 21, pp. 1661-1664.

COLLARD, D. AND CHARTIER, M., 1993. Biological decontamination of heavy metal polluted sediments. *Environ. Technol.*, **14**: 919-930.

HOLMES, D.S., 1984. Improved rapid heating technique for screening recombinant DNA plasmids in *E. coli.*, *Biotechniques*, **2**: 68-69.

KUSELL, M., LAKE, L., ANDERSSON, M. AND GERSCHENSON, L.E., 1978. Cellular and molecular toxicology of lead. II. Effect of lead on δ-amino levulinic acid synthetase of cultured cells. *J. Toxicol. Environ. Health*, **4**: 515-525.

LEE, L.H., LUSTIGMAN, H., CHU, I. AND YU, HSU. S., 1992. Effect of lead and cobalt on the growth of *Anacystis nidulans*. *Bull. Environ. Contam. Toxicol.*, **48**: 230-236.

MERGEAY, M. 1991. Towards an understanding of the genetics of bacterial metal resistance. *Trends Biotech.*, **9**: 17-24.

NIES, D.H. AND SILVER, S., 1995. Ion efflux systems involved in bacterial metal resistance. *J. Ind. Microbiol.*, **14**: 186-198.

SACHS, H.K., 1978. Intercurrent infections in lead poisoning. *Am. J. Dis. Child.*, **32**: 315-316.

SILVER, S., 1994. Exploiting heavy metal resistance systems in bioremediation. *Res. Microbiol.*, **145**: 61-66.

TONDWALKAR, S.V., NANDAN, R., AHMAD, S. AND KUMAR, R.P., 1990. Biomethanation of spent wash. Bacterial pre-treatment to remove heavy metals by adsorption. *J. Ferment. Bioeng.*, **69**: 302-304.

VERMA, S. K. AND SINGH, S.P., 1995. Multiple metal resistance in the cyanobacterium *Nostoc muscorum*. *Bull. Environ. Contam. Toxicol.*, **54**: 614-619.

WOOD, J. M. AND WANG, H.K., 1985. Microbial resistance to heavy metals. *Environ. Sci. Technol.*, **17**: 585-590

(Received: January 15, 1997)