Bull. Environ. Contam. Toxicol. (1987) 38:1049–1054


Environmental Contamination and Toxicology

# Bioaccumulation Potential of Heterotrophic Bacteria for Lead, Selenium, and Arsenic

G. A. Thompson[1] and R. J. Watling[2]

[1]Department of Pharmacy, University of Port Elizabeth, P.O. Box 1600, Port Elizabeth 6000, South Africa and [2]Pye Unicam Ltd., Cambridge, England

Metal compounds are essential for the development and survival of all life forms and bacteria play a major role in the geochemical and biological recycling of these compounds (Förstner and Muller 1976). In the marine environment, one of the most significant metal-microbe interactions is the uptake of metal compounds by bacteria. Such metal-bacterial bioaccumulations can be beneficial, as well as detrimental, to the ecology as a whole or to individual members of the biota. Positive effects include detoxification in the environment with consequent benefit to more susceptible species (Gadd and Griffiths 1978). Conversely, transfer of metals to high trophic levels (Patrick and Loutit 1976) can result in an increased range of toxic effects. At the microbial level changes in species diversity may reflect negatively on ecological stability (Mills and Colwell 1977). In addition the linkage between metals, drug resistance and plasmids (Nakahara et al. 1977) has significance for pollution control.

Fundamental studies on the ability of bacteria to bioaccumulate metals have not been extensively undertaken although this type of study is an essential aspect of pollution investigations. Such studies are also critical to the development of biological systems for use in toxic waste removal (Dunn and Bull 1983) as well as for the optimisation of recovery processes for the metals of industrial and commercial value.

This paper describes two simple experiments which were designed to obtain information on the potential of marine bacteria to bioaccumulate lead, selenium and arsenic.

## MATERIALS AND METHODS

Sediment was removed from the Swartkops Estuary and Papenkuils River in the Eastern Cape, South Africa, using sterile PVC corers (360 mm x 20 mm). Samples were taken to a depth of 100 mm. Two cooked meat media (Oxoid CM81) were inoculated for each sample, one incubated at 20°C for 48 h, the other at 37°C for 18 h.

Send reprint requests to G. A. Thompson at Leather Research Inst., P O Box 185, Grahamstown, South Africa

Sub-cultures were made of each inoculated media onto both MacConkey agar (M) (Oxoid CM115) and Nutrient agar (N) (Oxoid CM3) and these were incubated at 37°C (M) and 20°C (N). Pure cultures of bacterial growth were stored on Tryptone soya agar (Oxoid CM131) prior to identification with API 20E where applicable.

To determine metal uptake by bacteria, tryptone glucose broth (0.1% tryptone, 0.1% glucose, 0.5% NaCl) was prepared in 50 mℓ aliquots. Stock metal solutions were added to achieve final concentrations, as individual elements, of 30 µg Pb/mℓ ($PbCl_2$); 25 µg Se/mℓ ($SeO_2$); 100 µg As/mℓ ($Na_2AsHO_4$). In each case final metal content of the broth was just below the MIC value as determined by the agar diffusion method of Thompson and Watling (1983. 1984). Two strains of each of the following genus Bacillus, Pseudomonas, Klebsiella, Escherichia, Aeromonas and Enterobacter were inoculated on MacConkey agar and nutrient agar to determine purity. A few colonies from each plate were inoculated into 1% tryptone water (30°C 18 h) to obtain vigorously growing pure cultures of each strain selected. 0.5 mℓ of each of these cultures was added to each of the metal-amended media and to tryptone-glucose broth without metals. After incubation each broth was filtered through preweighed Millipore 45 µm filters prior to drying and reweighing. The filter and residue were digested with 5 mℓ concentrated nitric acid and allowed to evaporate to dryness on a hot plate. Five millilitres of 10% v/v nitric acid were added to the cooled residue and the samples left in sealed vials prior to the determination of metal content by atomic absorption spectroscopy (AAS).

To estimate bacterial/metal mass in the initial experiment, Millipore 45 µm (45 mm) filters were weighed after drying at 50°C for 3 h and cooling by desiccation at 20°C for one hour. Each metal-amended medium, both with and without bacteria, was filtered, using a sterile Seitz filter apparatus, through a preweighed filter. The filters were then reweighed, after drying at 20°C followed by 3 h drying at 50°C and cooling under dessication (20°C for one hour).

For the second bioaccumulation experiment the estimation of metal uptake by bacteria was carried out as follows: 120 mℓ of a broth containing 0.1% tryptone and 0.1% glucose in 25% filtered sea water was prepared. Lead chloride solution was added to give a final concentration of 30 µg Pb/mℓ. The test was undertaken in duplicate.

An overnight culture of E. coli (1.2 mℓ) in 1% tryptone broth was added to each broth. After incubation at 30°C for 5 days the cultures were washed by centrifuging at 3 000 rpm for 10 min. This was repeated twice using 25% sterile sea water as the washing fluid. Five millilitres of deposit was digested with concentrated

nitric acid. After evaporation and cooling the residue was dissolved in 5 mℓ 10% v/v nitric acid prior to metal concentration determination by AAS. An uninoculated control was included and this was treated as for the inoculated media.

To estimate bacterial biomass the washed deposit of the inoculated metal-amended media was pooled and mixed. Dilutions were made using 25% sterile sea water to give final dilution factors of $10^3$ to $10^8$/mℓ. MacConkey agar plates were surface spread with 0.1 mℓ of each diluted solution prior to incubation at 37°C for 18 h.

The tests were undertaken in triplicate. Dilutions, showing between 30 to 300 colonies per plate, were used to estimate the total viable count. The total biomass was estimated from the formula (Rodina 1972):

$$\frac{0.25\ \pi\ d^2 h \times t}{1\ 000} = M$$

where d = average width on one bacterium (taken arbitrarily as 0.5 µm)
h = average length of one bacterium (taken arbitrarily as 3.0 µm)
t = number of thousands of cells/mℓ
M = biomass in mg/ℓ

RESULTS AND DISCUSSION

The results of the metal uptake experiments (lead, selenium, arsenic) by heterotrophic sediment bacteria are shown in Table 1. High levels of accumulation by most bacterial species was evident for selenium and lead, but not for arsenic.

The masses of the bacteria/metal residues varied (Table 2) and must be assessed according to the amount of metal accumulated as well as its relative mass.

From these results *E. coli* (strain No. 8) was chosen for a subsequent, more defined, test to measure the uptake of $PbCl_2$.

The results of the second bioaccumulation experiment are shown in Table 3. They reflect the ability of the *E. coli* strain to accumulate large quantities of lead from the surrounding media. The results shown are average of duplicate tests.

The metal compounds used in this study (lead, selenium, arsenic) were selected on the basis that they are frequently encountered in industrial and domestic wastes and agricultural run-off respectively. The bacteria were selected to include possible autochthonous environmental isolates as well as enteric strains since these often co-exist in marine sediments.

Table 1. Uptake of lead, selenium and arsenic by heterotrophic bacteria.

| Species | Strain | Total metal uptake (μg) Pb* | Se* | As* |
|---|---|---|---|---|
| Klebsiella oxycota | 1 | 52.5 | 80.0 | 2.0 |
| | 2 | 710.0 | 130.0 | 0.5 |
| Bacillus sp. | 3 | 35.5 | 6.0 | 0.6 |
| | 4 | 40.5 | 20.0 | 0.3 |
| Pseudomonas sp. | 5 | 37.0 | 600.0 | 1.0 |
| | 6 | 83.0 | 155.0 | 0.6 |
| Escherichia coli | 7 | 735.0 | 97.5 | 1.1 |
| | 8 | 740.0 | 115.0 | 0.4 |
| Aeromonas hydrophila | 9 | 565.0 | 60.0 | 0.8 |
| | 10 | 545.0 | 110.0 | 0.4 |
| Enterobacter cloacae | 11 | 156.0 | 425.0 | 1.1 |
| | 12 | 166.0 | 240.0 | 3.6 |

*Total input was 1,500 μg Pb; 1,250 μg Se; and 500 μg As as per test

Table 2. Mass (mg) of bacteria + metal residues

| Strain No | Media | Media + As | Media + Se | Media + Pb |
|---|---|---|---|---|
| 1 | 0.6 | 2.0 | 0.1 | 1.8 |
| 2 | 0.1 | 0.1 | 0.8 | 2.3 |
| 3 | 0.1 | 0.2 | 0.1 | 0.1 |
| 4 | 0.1 | 0.7 | 0.1 | 0.1 |
| 5 | 2.0 | 2.1 | 2.8 | 2.3 |
| 6 | 2.0 | 1.5 | 1.6 | 2.1 |
| 7 | 0.3 | 1.1 | 0.9 | 2.4 |
| 8 | 1.6 | 0.2 | 0.7 | 2.4 |
| 9 | 0.8 | 2.2 | 0.9 | 1.6 |
| 10 | 0.9 | 0.5 | 0.6 | 1.1 |
| 11 | 3.6 | 1.6 | 1.4 | 1.0 |
| 12 | 1.3 | 2.6 | 1.9 | 0.9 |

Table 3. Bacterial biomass and lead content of washed cells of E. coli after incubation in lead-amended media.

| Pb content (mg/mℓ) | Total viable count (mℓ) | Biomass (mg/mℓ) | Pb. concentration mg/mg dry bacterial mass |
|---|---|---|---|
| 0.0395 | $88 \times 10^5$ | 0.0052 | 7.6 |

The metal uptake experiments demonstrated the ability of gram negative heterotrophic sediment bacteria to accumulate lead and selenium. The amount of metal accumulated varied with bacterial species but results from these preliminary tests indicated that from the original metal input there was a 49% uptake of lead by E. coli and a 48% uptake of selenium by Pseudomonas species. The average uptake for lead was 21.5% for all the bacterial strains tested and 25.3% for the Gram negative strains only. In the case of selenium an average of 13.6% uptake by all the bacterial species was found and this was increased to 16.1% when only the Gram negative results were used in the calculation. The apparent lack of accumulation by arsenic may be due to the formation of volatile arsenic compounds (methylarsines) (Klumpp 1980) and subsequent loss into the atmosphere.

The first experiment demonstrated that bacteria capable of bioaccumulating metals can be readily isolated from marine sediments. In the second experiment dry mass, as a measure of biomass, was discarded in favour of total viable count x cell volume. This was not an absolute measure, since an average size bacterium was an arbitrary figure only, based on the value expected for E. coli under the conditions of test. Also a capsulated strain of E. coli was used in this second experiment and glucose added to the growth media to promote capsular development. The results clearly indicate the ability of selected heterotrophic bacteria to accumulate lead (7.6 mg/mg bacterial dry mass). It is postulated that much of this accumulated lead is held in the capsular material (probably in a capsule, cell, metal matrix) rather than directly in the cells themselves. Gadd & Griffiths (1978) indicated that metal uptake by bacteria may be non-specific e.g. binding onto cell surfaces, slime layers, capsules, etc., or may involve metabolism-dependent transfer. While the latter may result in a greater uptake of metal per cell the former may yield greater recovery of metals. The implications for the increased use of bacteria, either in metal ore leaching processes or metal removal from toxic effluents are apparent. Whilst this study used pure cultures for measuring the bioaccumulation potential of selected bacterial species, in situ experiments are also needed in order to fully understand the environmental impact of such interactions. Nevertheless microcosm experiments are valuable in determining the interactions of a wide range of metal compounds and bacterial species. The results can then be applied to more detailed studies including the metal leaching potential of bacteria as well as the environmental effects of bioaccumulated metal compounds.

REFERENCES

Dunn GM, Bull AT (1983) Bioaccumulation of copper by a defined community of activated sludge bacteria. Eur J Appl Microbiol Biotechnol 17:30-34.

Förstner U, Muller G (1976) Association of metals in estuarine environments. Unesco Workshop on the Biochemistry of Estuarine Sediments. Melreux, Belgium, pp 1-9.

Gadd GM, Griffiths AJ (1978) Micro-organisms and heavy metal toxicity. Micro Ecol 4:303-317.

Kelly DP, Norris PR, Brierly CL (1979) Microbial methods for the extraction and recovery of metals. In: Microbial Technology, Current State, Future Prospects. Proc 29th Symposium Gen Microbiol (Eds. Bull AT, Ellwood DC and Ratledge C), Cambridge Univ Press, Cambridge, pp 263-308.

Klumpp DL (1980) Characteristics of arsenic accumulation by the sea weeds Fucus spiralis and Ascophyllum nodosum. Mar Biol 58:257-264.

Mills AL, Collwell RR (1977) Microbiological effects of metal ions in Chesapeake Bay water and sediments. Bull Environ Contam & Toxicol 18:99-103.

Nakahara H, Ishikawa T, Sarai Y, Kondo I, Kozukwe H, Silver S (1977) Linkage of mercury, cadmium, arsenate and drug resistance in clinical isolates of Pseudomonas aeruginosa. Appl & Environ Micro 33:975-976.

Patrick FM, Loutit M (1976) Passage of metals in effluents through bacteria to higher organisms. Wat Res 10:333-335.

Rodina AA (1972) Methods in aquatic microbiology. (Eds Colwell RR, Zambruski MS), Univ Park Press, p 161.

Thompson GA, Watling RJ (1983) A simple method for the determination of bacterial resistance to metals. Bull Environ Contam & Toxicol 31:705-711.

Thompson GA, Watling RJ (1984) Comparative study of the toxicity of metal compounds to heterotrophic bacteria. Bull Environ Contam Toxicol 33:114-210.

Received September 26, 1986; accepted November 24, 1986.