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Mini review

Bacterial interactions with silver

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Summary. This review examines interactions between bacteria and the biologically non-essential metal, silver. Aspects of silver toxicity, tolerance and accumulation (possible binding and uptake as opposed to energy-dependent transport) in bacteria are discussed. In addition, plasmid biology is examined briefly since little information is available on the exact mechanism(s) of plasmid-endoced silver resistance in bacteria.

Key words: Silver - Nickel - Bacteria - Toxicity - Metal tolerance - Accumulation

Introduction

The interactions of microorganisms with metals, particularly heavy metals, have been well documented (Hughes and Poole 1989). Furthermore, metal contamination of soil and aquatic systems has long been a priority environmental concern. Numerous studies have used selected algal strains as biomonitors of metal pollution in lakes, rivers and oceans as well as in investigations on the effects of metal mixtures (Wong et al. 1982, 1978) and specific metal toxicity (Baker et al. 1983; Trevors et al. 1986; Wong 1987). Algal strains displaying tolerance to polluting metal(s) in these environments have also been observed (Stokes et al. 1973).

The response of soil microorganisms exposed to heavy metals has been used less frequently as a toxicity monitor. Although metal deposition occurs in both soil and aquatic systems, sediment is often a sink for the pollutants. Bacteria have been used in numerous physiological and genetic studies of metal sorption, toxicity, accumulation, resistance and detoxification (Beveridge 1989; Landeen et al. 1989; Mullen et al. 1989; Silver and Misra 1988; Trevors 1989; Williams and Silver 1984). A sound knowledge of these types of interactions is fundamental to understanding the behaviour and fate of metal species in the environment. As the mechanisms of these interactions become better understood, they may have a potential role in the *in situ* decontamination of metal-polluted waste sites. At present, it is still questionable whether bacterial strains will be useful in bioremediation from both economic and practical viewpoints. This paper reviews bacterial interactions with silver, a non-essential element which has been widely used for jewelry, electroplating, photographic and medicinal purposes.

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Silver toxicity

The toxicity of silver to bacterial cells is well documented and has led to its use in treatment of burns in patients and in disinfection of water systems (Merck Index, 1983; Yahya et al. 1990). Silver ions at 15 mM have been found to inhibit oxidation of glucose, glycerol, fumarate, succinate, D- and L-lactate and endogenous substrates by intact *Escherichia coli* cells (Bragg and Rainnie 1973). These researchers showed that silver ions inhibited the respiratory chain at two sites: between *b* cytochromes and cytochrome *d* and between the site of substrate entry into the respiratory chain and flavoprotein in the NADH and succinate dehydrogenase regions.

At 20 μM, silver nitrate inhibited uptake of inorganic phosphate and caused efflux of accumulated phosphate (Schreurs and Rosenberg 1982). In addition, leakage of mannitol, succinate, glutamine and proline was induced. Further research led to the postulate that silver exerted its toxicity at more than one site, resulting in metabolite leakage. A likely mechanism of toxicity was proposed to be the collapse of cellular energy resources to a level where concentration gradients could no longer be maintained. Potential targets for silver include interference with energy-yielding reactions of the respiratory chain, collapse of the proton motive force and interference with phosphate uptake (Schreurs and Rosenberg 1982). Ghandour et al. (1988) suggested that silver toxicity may be generated indirectly through for-

mation of a silver salt resulting in chloride limitation to bacterial cells. A more direct mechanism of toxicity could be binding of silver to sites on proteins and enzymes (Ghandour et al. 1988).

Interference (such as competition for binding sites on surfaces of bacterial cells) with silver by other metal ions, such as copper, has been reported to reduce overall silver toxicity and may provide clues to its toxic mechanism(s). It has been proposed that silver and copper ions compete for sites at which silver exerts its toxic effects. For example, 0.66 μM Ag^+ exerted a smaller toxic effect on *Escherichia coli* K12 when 2.16 μM Cu^{2+} was also present (Ghandour et al. 1988). Hence, silver toxicity may be dependent on the $[Ag^+]/[Cu^{2+}]$ ratio rather than $[Ag^+]$ alone. It is possible that silver competes for cellular entry by an essential copper transport system. This competition results in an antagonistic response which may protect the cells from silver toxicity if the concentrations are not lethal to the entire bacterial population.

Silver accumulation

Bioaccumulation of silver by bacteria has been reported, although a specific uptake mechanism has not been established. Charley and Bull (1979) isolated a bacterial community that exhibited an adaptive tolerance to silver. Concentrations as high as 100 mM silver were tolerated when the bacterial community was pre-exposed to silver. When pre-grown in the absence of silver, tolerance was reduced. This bacterial community was reported to accumulate silver to concentrations of over 300 mg Ag^+/g dry biomass, at a rate of 21 mg $Ag^+ \cdot h^{-1} \cdot (g\ biomass)^{-1}$ (Charley and Bull 1979). The community consisted of three bacterial strains of which one strain, *Pseudomonas maltophilia,* appeared to be primarily responsible for high silver tolerance and accumulation. Combined tolerance of the community was somewhat higher than *P. maltophilia* alone. This demonstrated the importance of multispecies' interactions as well as individual species in determining the combined tolerance of the community. For example, one species may provide necessary nutrients for another species that displays higher tolerance (Charley and Bull 1979). These researchers also hypothesized that, since rates of silver bioaccumulation were inhibited as external silver concentrations increased, the accumulation may be dependent on respiration which is silver-sensitive.

Strains of *Thiobacillus* that accumulated excess amounts of silver from sulphide minerals have been described (Pooley 1982). Electron micrographs of these cells revealed that the bacterial membrane was covered with silver sulphide. As much as 25% of the bacterial biomass recovered after leaching of a silver sulphide mineral consisted of silver (Pooley 1982).

Goddard and Bull (1989a) studied the ability of growing and non-growing cultures of *Citrobacter intermedius* B6, isolated from sludge, to accumulate silver. They found that growing bacteria accumulated silver in dense deposits on the cell envelope. It was the opinion of these researchers that, in growing cultures, it was unlikely that silver accumulation was simply due to surface adsorption. This view is consistent with the report of Charley and Bull (1979). It was observed that cells harvested during mid- or late-exponential growth phase exhibited the highest levels of silver accumulation, approximately 4.35% (by mass) from an external concentration of about 2111.2 μmol Ag^+/l (Goddard and Bull 1989a). Accumulation occurred in a linear manner with increasing external silver concentrations. Although growing cells exhibited higher levels of silver accumulation below 200 μmol Ag^+/l (Goddard and Bull 1989a), it was concluded that an actively growing culture would be appropriate for silver accumulation only when low silver levels were to be recovered. Other problems associated with removal of silver from solutions by bacteria were also raised by these authors. First, accumulation may not occur uniformly throughout the growth cycle and this may lead to inaccurate estimates of uptake capacities. Second, heterogeneous uptake by members of the population may lead to decreased silver concentrations in the supernatant fluid which would encourage growth of less resistant members of the population. A third consideration is the ability of organic components of growth media to bind silver.

The composition of the growth medium has been reported to influence metal resistance, uptake and toxicity by affecting the availability of silver. For example, at a high NaCl concentration (1% mass/vol.), toxic Ag^+ ions were found to be removed from solution by forming insoluble AgCl (Gadd et al. 1989). Further evidence that the presence of NaCl reduced the biological availability of silver was provided when silver accumulation was found to be the lowest in *Pseudomonas stutzeri* cells grown in a medium supplemented with NaCl (Gadd et al. 1989). These results are predictable because of the known interaction between silver and Cl ions. However, when initially isolating silver-resistant bacterial strains, NaCl is often present in the agar medium. These researchers also found that cells grown in the presence of silver recovered and reached control-grown biomass levels after a lag period of 24 h. This may signify the occurrence of one or a combination of processes: the expression of an inducible resistance mechanism, the selection of resistant cells, or a reflection of the time period of silver detoxification by medium components (Gadd et al. 1989). In contrast to earlier work on multispecies by Charley and Bull (1979), *P. stutzeri* cells pre-grown in the presence or absence of $AgNO_3$ displayed only a small difference in their uptake capacities (Gadd et al. 1989). Similar results for *E. coli* were reported by Schreurs and Rosenberg (1982).

Silver uptake by resting cells of *P. stutzeri* occurred rapidly within the first minute of incubation (Gadd et al. 1989). This was attributed to non-specific binding of the metal to negatively charged groups on cell surfaces or within cells (Gadd et al. 1989). Levels of silver accumulated were relatively low by *P. stutzeri* (compared to sensitive strains) which is a reflection of its resistant ca-

pacity. Ghandour et al. (1988) demonstrated that silver accumulation by non-growing *E. coli* cells was due to both surface binding and intracellular uptake. These researchers proposed that silver was accumulated by an energy-independent process and ultimately bound at specific sites within the cell. Once these specific sites were saturated, binding to the cell surface occurred. Therefore, metal accumulation may occur in two stages: a rapid, reversible and metabolically independent surface binding, followed by a metabolically dependent irreversible intracellular accumulation.

Because resistant strains may be useful in the recovery of silver from waste sites, there is considerable interest in bacterial mechanisms of resistance which enable cells to resist higher and more toxic silver levels. Goddard and Bull (1989b) isolated silver-resistant Enterobacteriaceae strains, which were also resistant to nickel, from sewage and photographic processing effluent. *P. stutzeri* AG259 is an example of an organism that possesses silver resistance (Haefeli et al. 1984; Gadd et al. 1989). Presentation of evidence for the uptake and accumulation of silver often implied a close association of these abilities with resistance and detoxification. It is difficult, if not impossible, to discuss one aspect without the other. In order to prevent accumulation of toxic levels of silver, some mechanism of decreasing uptake of the metal may exist. As stated by Gadd et al. (1989), unless some type of intracellular detoxification mechanism exists, it appears most likely that resistant cells exclude or efflux silver. It is known that some *E. coli* and *P. stutzeri* strains referred to as silver-sensitive generally accumulate much higher levels of the metal than those which are considered resistant (Gadd et al. 1989; Starodub and Trevors 1990).

Initial evidence for a plasmid-encoded mechanism of silver tolerance was reported by Charley and Bull (1979), whereby the silver uptake capacity in *P. maltophilia* was lost when grown in a medium without silver. This observation suggests that the resistance was encoded on a plasmid which was spontaneously cured in the absence of silver selection. Plasmid isolation performed on *P. stutzeri* AG259 revealed the presence of a large plasmid (pKK1, 49.4 MDa) encoding silver resistance (Haefeli et al. 1984). This plasmid was transferred to a strain previously designated as being sensitive and, after culturing, exhibited silver resistance. The exact mechanism of this resistance is not known. It was proposed that some resistant pseudomonads reduced Ag^+ to Ag^0; but this reductive capacity also existed in sensitive strains so it remains unclear whether this was a consequence of resistance (Haefeli et al. 1984).

Recent work with other strains also suggests that the mechanism of silver resistance may be plasmid-encoded. A silver-resistant *E. coli* R1 strain grew without an extended lag phase in the presence of 1.0, 0.5, and 0.3 mM $AgNO_3$, and preliminary evidence indicated that the resistance was encoded on the 83-kb plasmid pJT1 (Starodub and Trevors 1989). Electron microscopy and energy-dispersive X-ray analysis showed the resistant strain did not accumulate silver, compared to a plasmid-cured sensitive strain, during active growth. Resting cells, however, did not exhibit silver resistance. These researchers proposed that although silver is not a required metal, it may gain access to the cell by means of a transport system for an essential metal. Further evidence discouraged the possibility that the resistance mechanism was linked to an enzymatic transformation. Finally, although the production of H_2S was observed in both the sensitive and resistant strains, it was unclear whether *E. coli* R1 could overproduce H_2S as a detoxification mechanism (Starodub and Trevors 1989; 1990).

Nakahara et al. (1989) isolated silver-resistant strains of *Enterobacter cloaceae* from contaminated sewage. Their work demonstrated the existence of R plasmids with Ag^+ resistance through the ability to transfer that resistance. A ratio of minimum inhibitory Ag^+ concentrations of resistant/sensitive strains was at least 1000:1.

It has been postulated that the level of resistance depends on silver-complexing components such as halide ions. One hypothesis suggested sensitive cells bound Ag^+ tightly enough to extract it from AgCl and other complexed forms (Silver and Misra 1988). However, cells with Ag^+-resistance plasmids do not successfully compete with other precipitates for Ag^+. Haefeli et al. (1984) postulated the existence of a metallothionein that protects cells by binding and inactivating Ag^+. However, there is no experimental support for this hypothesis (Starodub and Trevors 1990).

This review of bacterial responses to silver exposure has shown evidence of uptake and accumulation of the metal which may be related to its extreme toxicity. However, despite its toxicity (Trevors 1987), resistant bacterial strains have been isolated, and preliminary work suggests plasmid-encoded resistance mechanism(s) in selected strains. The uptake of a non-essential metal such as silver by both sensitive and resistant strains is not well understood (Trevors 1987) and mechanism(s) of resistance remains largely uncharacterized.

From a biotechnology perspective, little information is available on the usefulness of selected bacterial strains in efficient, cost-effective silver recovery. The idea of using bacterial strains to recover silver from effluents or in mining operations has existed for decades. However, from a practical point of view, several factors must be considered. For example, silver-resistant bacterial strains often exclude this metal from accumulating in cells. This results in an overall decrease in silver accumulation. Hence the silver is not trapped by the cells where it can be recovered. Conversely, bacterial strains that accumulate silver are not tolerant to this metal and will take up silver in a very short period of time (minutes). If the silver concentration is toxic, the cells may lyse or become non-viable. Therefore, silver-sensitive strains may not be of any biotechnological value in cost-effective silver recovery. A silver-resistant strain that could accumulate high levels of silver and survive under the conditions used in the recovery process may have the most promising potential. For example, Pooley (1982) reported on the accumulation of silver by bacterial cells during leaching of sulphide ore minerals. However, the exact mechanism of this accumulation

and its relationship to silver resistance are not well understood. Moreover, if silver-accumulating strains are to be used in recovery processes, it may be difficult to maintain the viability of microbial cell numbers and a suitable cell density to ensure efficient recovery of the metal.

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References

Baker MD, Inniss WE, Mayfield CI, Wong PTS, Chau YK (1983) Effect of pH on the methylation of mercury and arsenic by sediment microorganisms. Environ Tech Lett 4:89–100

Beveridge TJ (1989) Role of cellular design in bacterial metal accumulation and mineralization. Annu Rev Microbiol 43:147–171

Bragg PD, Rainnie DJ (1973) The effect of silver ions on the respiratory chain of *Escherichia coli*. Can J Microbiol 20:883–889

Charley RC, Bull AT (1979) Bioaccumulation of silver by a multispecies community of bacteria. Arch Microbiol 123:239–244

Gadd GM, Laurence OS, Briscoe DA, Trevors JT (1989) Silver accumulation in *Pseudomonas stutzeri* AG259. Biol Metals 2:168–173

Ghandour W, Hubbard JA, Deistrung J, Hughes MN, Poole RK (1988) The uptake of silver ions by *Escherichia coli* K12: toxic effects and interaction with copper ions. Appl Microbiol Biotechnol 28:559–565

Goddard PA, Bull AT (1989a) Accumulation of silver by growing and non-growing populations of *Citrobacter intermedius* B6. Appl Microbiol Biotechnol 31:314–319

Goddard PA, Bull AT (1989b) The isolation and characterization of bacteria capable of accumulating silver. Appl Microbiol Biotechnol 31:308–313

Haefeli C, Franklin C, Hardy K (1984) Plasmid-determined silver resistance in *Pseudomonas stutzeri* isolated from a silver mine. J Bacteriol 158:389–392

Hughes MN, Pole RK (1989) Metals and micro-organisms. Chapman and Hall, London. pp 1–412

Landeen LK, Yahya MT, Gerba CP (1989) Efficacy of copper and silver ions and reduced levels of free chlorine in inactivation of *Legionella pneumophila*. Appl Environ Microbiol 55:3045–3050

Mullen MD, Wolf DC, Ferris FG, Beveridge TJ, Flemming CA, Bailey GW (1989) Bacterial sorption of heavy metals. Appl Environ Microbiol 55:3143–3149

Nakahara H, Yonekura I, Sato A, Moriyama K, Kobayashi Y, Mori T, Chino M (1989) Plasmid-determined resistance to silver in *Enterobacter cloacae* isolated from sewage. Water Sci Tech 21:275–278

Pooley FD (1982) Bacteria accumulate silver during leaching of sulphide ore minerals. Nature 296:642–643

Schreurs WJ, Rosenberg H (1982) Effect of silver oins on transport and retention of phosphate by *Escherichia coli*. J Bacteriol 152:7–13

Silver S, Misra TK (1988) Plasmid-mediated heavy metal resistances. Annu Rev Microbiol 42:717–743

Starodub ME, Trevors JT (1989) Silver resistance in *Escherichia coli* R1. J Med Microbiol 29:101–110

Starodub ME, Trevors JT (1990) Silver accumulation and resistance in *Escherichia coli* R1. J Inorg Biochem 39:317–325

Stokes PM, Hutchinson TC, Krauter K (1973) Heavy-metal tolerance in algae isolated from contaminated lakes near Sudbury, Ontario. Can J Bot 51:2155–2168

The Merck Index: An encyclopedia of chemicals, drugs, and biologicals (1983) 10th edn, Windholz et al. (eds). Merck & Co, Rahway NJ

Trevors JT (1987) Silver resistance and accumulation in bacteria. Enzyme Microb Technol 9:331–333

Trevors JT (1989) The role of microbial metal resistance and detoxification mechanisms in environmental bioassay research. Hydrobiologia 188/189:143–147

Trevors JT, Stratton GW, Gadd GM (1986) Cadmium transport resistance, and toxicity in bacteria, algae, and fungi. Can J Microbiol 32:447–464

Williams JW, Silver S (1984) Bacterial resistance and detoxification of heavy metals. Enzyme Microb Technol 6:530–537

Wong PTS (1987) Toxicity of cadmium to freshwater microorganisms, phytoplankton, and invertebrates. In: Nriagu JO, Sprague JB (eds) Cadmium in the aquatic environment. John Wiley and Sons, New York, pp 117–138

Wong PTS, Chau YK, Luxon PL (1978) Toxicity of a mixture of metals on freshwater algae. J Fish Res Board Can 35:479–481

Wong PTS, Chau YK, Patel D (1982) Physiological and biochemical responses of several freshwater algae to a mixture of metals. Chemosphere 11:367–376

Yahya MT, Landeen LK, Messina MC, Kutz SM, Schultz R, Gerba CP (1990) Disinfection of bacteria in water systems by using electrolytically generated copper: silver and reduced levels of free chlorine. Can J Microbiol 36:109–116