Silver resistance in *Escherichia coli* R1

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**Summary.** *Escherichia coli* strain R1, originally isolated from a patient whose burns were treated with silver sulphadiazine, contained two large plasmids of 83 kb (pJT1) and 77 kb (pJT2), and was resistant to 1 mM AgNO₃. A silver-sensitive derivative, *E. coli* S1, cured of the 83-kb plasmid pJT1, was obtained by growth at 46°C. Studies with an Ag⁺-specific ion electrode showed no significant differences in Ag⁺ binding by washed resting cell suspensions of strains R1 and S1, with and without glucose. However, transmission electronmicroscopy and energy dispersive X-ray analysis of whole cell mounts from actively growing cultures showed that the Ag⁺-resistant strain did not accumulate Ag⁺, whereas the sensitive strain contained dense silver particles. Both strains produced H₂S, detected by blackening of lead acetate paper above inoculated broth, and reducing substances (possibly H₂S) were detected only around *E. coli* R1 colonies when methylene blue was used as a indicator in LB agar, which may be a less sensitive assay. The mechanism of silver resistance is not known, but actively growing cells of *E. coli* R1 did not accumulate silver.

**Introduction**

The soluble form of silver is the most toxic to bacteria (Tilton and Rosenberg, 1978; Trevors, 1987) and silver toxicity can be reduced by precipitation of the metal by phosphates, sulphides and chloride ions (Trevors, 1987). There are silver-resistant bacteria, such as *Thiobacillus ferrooxidans* and *T. thiooxidans*, that accumulate silver during the leaching of sulphide ore (Pooley, 1982) and it is also known that silver resistance can be plasmid-encoded in *Pseudomonas stutzeri* (Haefeli et al., 1984), *Citrobacter* spp. (Silver, 1981) and some members of the Enterobacteriaceae (Annear et al., 1976; Bridges et al., 1979).

Silver-resistant bacteria have been isolated and studied (Charley and Bull, 1979), but were not examined for the possibility that resistance was plasmid-encoded. Belly and Kydd (1982) isolated silver-resistant bacteria, yeast and fungi; resistant bacteria were able to tolerate 300 ppm of AgNO₃ whereas sensitive strains failed to grow in the presence of 1–10 ppm. Hendry and Stewart (1979) isolated silver-resistant Enterobacteriaceae from hospital patients. However, the mechanism of silver resistance is not understood and there is little information on silver uptake and silver binding to specific components in sensitive and resistant organisms (Trevors, 1987). We report here preliminary evidence that silver resistance in *Escherichia coli* may be plasmid encoded.

**Materials and methods**

**Bacterial strains and growth conditions**

The silver-resistant *E. coli* strain R1 was isolated from a patient whose burns had been treated with sulphadiazine (Hendry and Stewart, 1979) and maintained on LB agar (Acumedia, Baltimore, USA) containing 0.5 mM AgNO₃. Cultures were grown in 10 ml of LB broth for 12 h with shaking at 120 rpm. When AgNO₃ was used in broth or agar, a stock solution was added separately to give the desired concentration. Culture turbidity was measured spectrophotometrically at 650 nm. Tubes of LB broth were inoculated with a 1% v/v inoculum of mid-logarithmic phase cells grown in the absence of AgNO₃. *E. coli* strain C600 (F-, nalidixic acid resistant) and *E. coli* HB101 competent cells (Gibco/BRL, Burlington, Canada) were used as recipients in conjugation and transformation experiments, respectively.

**Plasmid isolation and curing**

Plasmid DNA was isolated by the alkaline-sodium dodecyl sulphate method of Jayaratne *et al.* (1987). Plasmids were visualised by staining for 1 h in an aqueous solution of ethidium bromide 0.5 μg/ml and transilluminated at 302 nm. Plasmid sizes were estimated by comparing their mobility in agarose gels with those of
reference plasmids from E. coli strains V517 (Macrina et al., 1978), MA527 (Trevors and Oddie, 1986) and 1784 (Gealt et al., 1985) by use of a Restriction Fragment Sizing and Sequence Entry Program (Molecular Software Inc., Atlanta, USA) operated on an IBM-PC microcomputer.

A silver-sensitive derivative (strain S1) cured of the 83-kb plasmid pJT1 was obtained by growing E. coli R1 at 46°C. Single colonies were replica plated from non-selective LB agar to LB agar with 0.5 mM AgNO₃. Colonies that failed to grow in the presence of 0.5 mM AgNO₃ were examined for the loss of plasmid DNA.

Silver binding by resting cell suspensions

Log-phase cultures (25 ml) in LB broth containing 20 mM glucose with 0.1 mM or 1.0 mM AgNO₃, or without AgNO₃, were centrifuged at 10 000 g for 10 min at 20°C. The cells were resuspended in 5 mM MES buffer (2[N-morpholino]ethanesulphonic acid, pH 6.5) centrifuged again and resuspended in the same buffer. In Ag⁺-binding studies, cell suspensions were starved for 2 h at 37°C to metabolise intracellular carbon sources before adding glucose, as recommended by Packer (1967). Ag⁺ concentrations in the resting cell suspensions were measured with an Orion 407A ion meter and silver-specific electrode (Orion Research Incorporated, Cambridge, MA, USA). MES buffer was used because it has negligible metal binding properties (Good et al., 1966; De Rome and Gadd, 1987). A double liquid junction reference electrode (Orion) was used in conjunction with the Ag⁺ electrode. A two-point calibration with AgNO₃ dissolved in MES buffer was used to standardise the meter. Glucose (when needed) and AgNO₃ were added to give final concentrations of 50 mM and 0.1 mM, respectively. Ag⁺ binding and accumulation was measured at 4°C and 37°C.

Oxygen consumption

The effect of silver on O₂ uptake by resting cell suspensions was measured as described by Trevors (1983). A 200-ml overnight culture of each strain grown with or without 0.1 mM AgNO₃ (non-lethal concentration) in LB broth at 37°C with shaking at 120 rpm was harvested by centrifugation, washed, and resuspended in 100 ml of 5 mM PIPES (piperazine-N, N-bis[2-ethanesulphonic acid] buffer, pH 6.5), and 9 ml of cell suspension was added aseptically to sterile 50-ml Erlenmeyer flasks. Sterile glucose was added to give a final concentration of 20 mM and AgNO₃ was added to give the required concentration; control flasks received an equal volume of sterile distilled water instead of AgNO₃. The flasks were sealed with sterile serum stoppers and incubated at 37°C with shaking at 120 rpm. At intervals, 0.2 ml of the gas phase was removed with a gas-tight syringe, and injected into the chromatographic system described by Trevors (1983). All experiments were performed in duplicate. Dry weights of cell suspensions were determined by filtering 10 ml of cell suspensions through 0.22-μm cellulose acetate filters which were dried at 105°C for 12 h and weighed.

Conjugation and transformation experiments

Filter conjugation matings were performed with E. coli C600 as the recipient by the method of Willetts (1984). Samples of the mating mixture were serially diluted and plated on LB agar (non-selective) and LB agar with 0.5 mM AgNO₃ (selective for possible transconjugants). Randomly selected colonies were inoculated into 5 ml of LB broth, grown for 12 h at 37°C, and examined for the presence of plasmid DNA by agarose gel electrophoresis.

Plasmid DNA used in transformation experiments was electroeluted from low-melting-point agarose after the gel had been heated at 65°C for 10 min in TE buffer to liquify the gel matrix. The solution was placed in an ISCO electrophoretic concentrator (Lincoln, NB, USA) operated at 100 V for 1 h. The polarity was then reversed for 10 s at 100 V to remove plasmid DNA from the membrane. Concentrated plasmid DNA (200 μl) was pipetted from the collection chamber and precipitated at −20°C for 12 h after adding 20 μl of 3 mM sodium acetate and 500 μl of isopropanol. The sample was centrifuged at 15 500 g for 30 min at 4°C, resuspended in sterile TE buffer and stored at −20°C until used in transformation experiments. For recovering plasmids from agarose gels, a 40 mM Tris-acetate, 1 mM ethylenediamine tetracetate buffer was used during the initial electrophoresis of the plasmid DNA and in the ISCO concentrator, because plasmid recoveries were usually higher than with Tris-borate buffer. Plasmid DNA was also recovered from low-melting-point agarose by phenol extraction (Nestmann et al., 1985).

E. coli HB101 cells were made competent and transformed by the method of Nestmann et al. (1985). Potential transformants were selected on LB agar with 0.5 mM AgNO₃.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

Whole cell and outer-membrane proteins were prepared from 200 ml of overnight cultures as described by Miura and Mizushima (1969). The outer-membrane samples were solubilised in Triton X-100 2% w/v by the method of Schnaitman (1971). Equal volumes (20 μl) of protein samples were analysed on vertical polyacrylamide 10% w/v gels in a Hoefer SE-600 apparatus by the procedure of Laemmli (1970). All experiments were repeated at least twice. Gels were stained with Brilliant Blue R and destained by diffusion. Protein scans were performed and molecular weights of proteins were estimated by comparing mobilities of unknown protein bands with standards (BRL, Burlington, Canada) by means of a Hoefer GS-300 densitometer (Technical Marketing Associates, Mississauga, Canada) controlled by an IBM-PC microcomputer.
Production of reducing substances

LB agar plates containing 0·1 mM AgNO₃ and methylene blue 0·002% w/v as a redox indicator were used to determine if reducing compounds were produced during the growth of strains R1 and S1. A positive test for reducing compounds is a clearing (reduction of methylene blue to colourless) around bacterial colonies (Belly and Kydd, 1982). All plates were incubated at 37°C for 1 week in the dark. Assay for volatile reducing compounds was by the inverted plate technique of Belly and Kydd (1982); an uninoculated agar plate containing methylene blue was inverted over an inoculated plate of LB agar with 0·1 mM AgNO₃ and the plates were sealed and incubated at 37°C.

The presence of H₂S was detected by lead acetate paper (Fisher Scientific, Toronto, Canada) suspended 1 cm above LB broth in serum-stoppered flasks, which prevented H₂S from escaping but provided enough O₂ in the headspace for growth. Kliglers Iron Agar (Difco) was also used to test for H₂S production (MacFaddin, 1976).

Transmission electronmicroscopy

A washed cell pellet of each organism grown with and without 0·1 mM AgNO₃ was resuspended in 500 μl of sterile distilled water and dropped on to an aluminium grid. Samples were air dried and examined in a Philips electronmicroscope equipped with an energy dispersive X-ray detector.

Results

Plasmid isolation and curing

The silver-resistant E. coli strain R1 contained plasmids of 83 and 77 kb (fig. 1, lane 1). The plasmid sizes were estimated by comparison with the reference plasmids shown in lanes 3 and 4 (fig. 1). The 83-kb plasmid pJT1 was cured by growing the organism at 46°C (fig. 1, lane 2). After curing of the 83-kb plasmid, the strain was sensitive to silver. This preliminary finding suggested that silver resistance was encoded by the plasmid.

Inhibition of growth by silver

E. coli strain R1 was able to grow in the presence of higher silver concentrations than the cured derivative, S1 (fig. 2); at AgNO₃ concentrations of 1, 0·5 and 0·3 mM, strain R1 cultures did not have an extended lag phase, but the final cell biomass was slightly reduced in the presence of any of the AgNO₃ concentrations (fig. 2). Strain S1 was capable of growth in the presence of 0·1 mM AgNO₃, but not at 0·5 or 1·0 mM (fig. 3), even when the incubation period was extended to 48 h. Plate counts of strain R1 on LB agar with 0·5 mM AgNO₃ showed that the cells were viable. Colonies of E. coli R1 growing in the presence of AgNO₃ were black after 24–48 h.

Fig. 1. Agarose (0·7% w/v) gel electrophoresis showing: lane 1, 83- and 77-kb plasmids isolated from Ag⁺ resistant E. coli strain R1; lane 2, strain S1 lacking the 83-kb plasmid pJT1; lane 3, 78-kb plasmid from E. coli reference strain 1784; lane 4, 87-kb plasmid from E. coli reference strain MA527. Chr= chromosomal DNA.

Fig. 2. Growth of E. coli R1 (containing pJT1 plasmid) in LB broth at 37°C with shaking: control, no AgNO₃ (○); with 1 mM AgNO₃ (▲); with 0·5 mM AgNO₃ (▲); with 0·3 mM AgNO₃ (●).
1.2 Silver binding

Binding of Ag⁺ occurred in <1 min in resting cell suspensions of strains R1 and S1 grown with or without 0-1 mM AgNO₃ (table I). Cells of strain R1 grown without AgNO₃ bound less Ag⁺ than cells grown with 0-1 mM AgNO₃ (table I); e.g., at 37°C in the presence of glucose, Ag⁺ binding was two-fold higher in cells grown in the presence of AgNO₃. Ag⁺ binding was not decreased by incubation at 4°C, nor increased in the presence of glucose at 37°C in cells grown in the presence of AgNO₃.

Cells grown without AgNO₃ did not initially bind as much Ag⁺ as cells grown in the presence of AgNO₃ (table I). Incubation at 4°C, or in the absence of glucose, did not significantly decrease the binding of Ag⁺ to cells which had been grown in the absence of AgNO₃. However, cells grown in the presence of AgNO₃ were capable initially of binding more Ag⁺. Moreover, cells of strain R1 grown in the presence of AgNO₃ bound more Ag⁺ than cells of strain S1 grown and assayed under identical conditions.

Oxygen consumption

O₂ consumption by resting cell suspensions of strains R1 and S1 grown without 0-1 mM AgNO₃ (a concentration not inhibitory to either strain) showed that both organisms were equally sensitive to AgNO₃ concentrations of 0-05-0-5 mM (table II).

However, resting cells of strain R1 that had been grown with 0-1 mM AgNO₃ had higher O₂-consumption rates in the presence of 0, 0-05 and 0-1 mM AgNO₃ than suspensions of strain S1 cells grown in identical conditions (table II). We cannot explain why the controls also exhibited higher respiration rates. Strain R1 consumed O₂ at 5-05 μmol/mg/h in the presence of 0-05 mM AgNO₃, whereas strain S1 consumed 1-0 μmol/mg/h (table I). However, in the presence of 0-5 mM AgNO₃, both strains displayed similar O₂ consumption rates, because this silver concentration was toxic even to the resistant strain in a buffered solution. Respiration by cells of strain R1 was less sensitive to Ag⁺ when cells had been grown in the presence of a non-toxic concentration of AgNO₃.

Conjugation and transformation experiments

Membrane filter matings with E. coli C600 as the recipient and strain R1 as the donor revealed that the 77-kb plasmid, but not the 83-kb plasmid pJT1, was transferred during conjugation (fig. 4, lane 3). Agarose gel electrophoresis showed that all of five randomly selected transconjugants contained one 77-kb plasmid. These transconjugants were not resistant to Ag⁺ in growth curve experiments in LB broth with 0-5 mM AgNO₃, indicating that the transferable 77-kb plasmid was not involved in Ag⁺ resistance. Antibiotic sensitivity testing with
**Table I.** O₂ consumption by resting cell suspensions of strains R1 and S1 grown with (Ag⁺) and without (Ag⁻) 0.1 mM Ag NO₃

<table>
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<tr>
<th>AgNO₃ concentration (mM) in test</th>
<th>R1 Ag⁺</th>
<th>R1 Ag⁻</th>
<th>S1 Ag⁺</th>
<th>S1 Ag⁻</th>
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* n = 2.

**Table II.** Ag⁺ binding by starved resting cell suspensions of strains R1 and S1 grown with (Ag⁺) and without (Ag⁻) 0.1 mM AgNO₃

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*(n = 3).
† No glucose present; + glucose present.
E. coli C600 and an E. coli transconjugant showed that the transconjugants became resistant to tetracycline and streptomycin when they acquired the 77-kb plasmid, i.e., these antibiotic resistance determinants were carried on the 77-kb conjugative plasmid.

**SDS-PAGE**

Densitometer scans of SDS-PAGE of whole cell extracts from strains R1 and S1 are shown in fig. 5 (sections B and C, respectively). Molecular weight standards from 14.3 to 200 Kda were used to estimate the sizes of unknown proteins (fig. 5, section A). Protein samples of strain R1 (fig. 5, section B) yielded 25 protein bands. A scan of samples from strain S1 treated in the same manner revealed one additional large protein band (between 68 and 97.4 Kda) and a smaller additional protein band (c. 68 Kda) were present. Outer-membrane protein samples showed that the same two protein bands were absent from strain R1 (densitometer

![Fig. 5](image-url)
scan not shown). This suggested that the proteins missing from strain R1 were located in the outer membrane of strain S1.

Production of reducing substances

Reducing compounds were detected by the fading of the methylene blue indicator when strain R1 was streaked on LB agar with 0·1 mM AgNO3 and methylene blue, and incubated for 1 week; the indicator plate slowly turned from an intense blue to a faint blue-brown. This is a qualitative test for the presence of reducing compounds and has been used by Belly and Kydd (1982) to study Ag+ resistance in a Pseudomonas sp. Methylene blue has a 50% \textit{E}_\text{u} value of +0·01 V. This represents the potential required to reduce 50% of the indicator, and is an estimate of reducing activity. In the absence of silver, no fading of the methylene blue, i.e., no reduction, was observed. The silver-sensitive strain S1 did not produce any reducing compounds and uninoculated control plates did not show any change in the indicator after 1 week.

H₂S production

 Cultures of strains R1 and S1 grown with and without AgNO₃ were assayed for H₂S production with lead acetate paper (MacFaddin, 1976). H₂S was produced by both organisms but production was not quantified. It is difficult to quantify H₂S production in the presence of metals because insoluble metal sulphide complexes are formed. H₂S production was not detected in the Kligler iron agar test.

Transmission electronmicroscopy (TEM)

 Electronmicroscopy (fig. 6) and energy dispersive X-ray analysis (fig. 7) revealed that Ag⁺ had not accumulated in cells of strain R1 where it could produce a toxic lethal effect. Silver deposition was not observed in whole cell preparations of strains R1 and S1 grown without AgNO₃ and examined by TEM. Silver deposits were observed throughout cells of strain S1 grown with AgNO₃ (fig. 6, section 2). Nearly all the cells of strain R1 examined did not contain electron dense silver particles (fig. 6, section 1). Energy dispersive X-ray analysis (fig. 7) revealed that the cells of strain R1 did not contain Ag⁺, whereas the cells of the sensitive strain S1 yielded a high Ag⁺ signal. When the same strains were grown in the absence of Ag⁺, silver was not detected in either cell preparations. When strain R1 was grown in the presence of 1·0 mM AgNO₃, most cells were viable and the Ag⁺ signal was still less than the signal from cells of strain S1 grown in the presence of 0·1 mM AgNO₃. It is noteworthy that the phosphorus (P) signal was relatively high in the resistant cells (fig. 7, section 1) and it is unlikely that the P signal was hidden by the increased silver signal in cells of strain S1. A decreased P signal and absent K signal were observed in the Ag⁺ sensitive strain S1, indicating leakage of these ions from the cells. Al, Fe and Cu from the mounting grid gave high background signals.

Discussion

Mechanisms of silver resistance in bacteria are not well understood (Trevors \textit{et al.}, 1985). Preliminary evidence from the present studies indicates that Ag⁺ resistance in \textit{E. coli} R1 was encoded on the 83-kb plasmid pJT1. Better evidence that Ag⁺ resistance was plasmid encoded would be transformation of \textit{E. coli} HB101 (or other recipient strain) with plasmid pJT1 and expression of Ag⁺ resistance, but \textit{E. coli} HB101 was not transformed with the plasmid. Control experiments with plasmid pBR322 DNA showed that the cells of \textit{E. coli} HB101 were competent and able to take up and express the antibiotic resistances encoded on plasmid pBR322. Because transformation of \textit{E. coli} HB101 was not achieved, an alternative procedure of transformation \textit{via} electroporation of the plasmid DNA into an Ag⁺-sensitive \textit{E. coli} recipient strain is currently being investigated.

Protein profiles were examined because McEntee \textit{et al.} (1986) reported that a new protein was induced in a Cd²⁺ resistant \textit{Klebsiella} strain grown in the presence of 0·1 mM Cd²⁺. However, instead of
detecting a new protein band in *E. coli* R1, we found that two proteins bands present in strain S1 were missing from strain R1. The missing bands may represent individual proteins or constellations of proteins whose mobilities give the appearance of single bands.

Accumulation of metals into bacterial cells usually requires specific transport systems (Brierley et al., 1985). Accumulation may occur via a two-stage process: (1) a rapid, metabolic-independent surface-binding to cells; followed by (2) a metabolic-dependent intracellular accumulation of the metal. Stage 1 is usually rapid, occurring in minutes, whereas stage 2 is gradual and may take from several minutes to some hours. Active transport, but not binding of metals, is usually inhibited at 4°C, and is also decreased in the absence of an energy source. About 50% of the Ag⁺ was removed from the assay solution during the first minute; this rapid removal was probably binding, followed by a gradual accumulation of the metal, indicative of stage 2 kinetics (Brierley et al., 1985). However, electronmicroscopy and energy dispersive X-ray analysis showed that Ag⁺ had accumulated in cells.

Fig. 7. Representative energy dispersive X-ray analysis of whole cell preparations of *E. coli* strains R1 (1) and S1 (2) grown in the presence of 0.1 mM AgNO₃. Energy (0-10-240 KeV) is the X axis. Vertical full scale (VFS) on Y axis is 0 to 512 peak units.
of strain S1, but not in strain R1 cells. This suggested that actively growing cells (not washed resting cells) of strain R1 could exclude Ag⁺ from the cells, but starved, washed resting cells of strain R1, assayed for Ag⁺ binding in a buffer without essential growth nutrients, were unable to exhibit Ag⁺ resistance. Ag⁺ is not an essential metal and it is unlikely that there is a specific energy dependent transport system for it, but, Ag⁺ could enter cells via a transport system for an essential metal.

The role of H₂S production in resistance to silver in not clear. E. coli R1 may produce more H₂S than strain S1, but it is also noteworthy that H₂S production was detected in cultures of both strains by the lead acetate test, although reducing compounds were not detected in strain S1 by the methylene blue assay. Moreover, H₂S production was not detected after 7 days in cultures of either strain in Kligler iron agar incubated at 37°C as described by MacFaddin (1976). H₂S production by the positive control strain, Proteus mirabilis (MacFaddin, 1976) was detected after incubation for 20 h at 37°C. These results are not conflicting, because the lead acetate test for H₂S is very sensitive and detects small quantities of H₂S (0-2 mM), whereas the Kligler iron agar test is less sensitive (MacFaddin, 1976).

Erardi et al. (1987) reported that a copper-tolerant strain of Mycobacterium scrofulaceum removed copper from culture media by precipitation as copper sulphide, but precipitation was not observed in a derivative strain lacking a 173-kb plasmid. An independent, but unknown, copper resistance mechanism was also present in M. scrofulaceum. A similar situation may exist in E. coli strain R1.

Pan-Hou and Imura (1981) have studied the role of H₂S production in resistance to mercury. They suggested that the genes conferring H₂S production and methylmercury decomposing activity were carried on a plasmid in Clostridium cochlearium T-2 but mercuric reductase activity was not detected in the strain. A plasmid-cured derivative of C. cochlearium T-2 could not produce H₂S or decompose methylmercury. Kane et al. (1975) reported that the his mutation in Bacillus subtilis results in overproduction and excretion of H₂S as detected by blackening of lead acetate paper placed in the top of inoculated culture tubes. Because H₂S is toxic to most bacteria, it would not be beneficial to overproduce H₂S unless the sulphide complexed with metals like silver, mercury, copper or lead to form insoluble precipitates. Such a mechanism would make the metal(s) less toxic and reduce the amount of toxic H₂S.

It has been suggested that the mechanism of Ag⁺ resistance may be similar to mercuric reductase activity found in some mercury-resistant bacteria (Charley and Bull, 1979; Belly and Kydd, 1982). Ag⁺-dependent oxidation of NADPH was measured spectrophotometrically at 340 nm in MES buffer with the protocol described by Izaki (1981). No Ag⁺-dependent oxidation of NADPH was observed in cell-free extracts of either strain prepared from cultures grown with or without AgNO₃. Furthermore, the presence of 2 mM 2-mercaptoethanol (SH source for Hg²⁺ reductase activity) in the assay mixture did not influence the reaction. This indicated that an enzymic transformation of Ag⁺, similar to that catalysed by the Hg²⁺ reductase enzyme, was probably not the resistance mechanism.

Silver ions can complex with electron donor groups containing sulphur, oxygen or nitrogen (Block, 1977). In biological systems, these elements are normally present as thiols, phosphates, hydroxyl amines and indoles (Block, 1977). Silver has a lower binding affinity for S⁻, NH₃, acetate and glycinate ions than mercuric ions, but low concentrations of Ag⁺ are still extremely toxic to many bacterial species. The mechanism of Ag⁺ resistance in E. coli strain R1 may be different from the plasmid-encoded Cd²⁺ efflux in Staphylococcus aureus and Hg²⁺ volatilisation in certain gram-negative and -positive bacterial species (Trevors et al., 1985) and it appears that bacterial species have evolved different mechanisms for tolerating these different toxic metals. Moreover, not all adaptations to metals are mediated through plasmids and there is evidence that cells can alter their biochemistry for adaptation to metals (Trevors et al., 1985; Trevors, 1987).

The results of our study suggest that silver-resistance may be plasmid encoded. E. coli strain R1 contained a 88-kb plasmid that was cured by growth at an elevated temperature, producing an Ag⁺ sensitive derivative that was unable to grow in the presence of 0-5 mM AgNO₃. Both strains produced H₂S, but it was not clear whether strain R1 could overproduce H₂S as a detoxification mechanism or whether there was another unknown mechanism of resistance. TEM confirmed that Ag⁺ was deposited in the cells of the sensitive strain S1 but not in the resistant strain R1.

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