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MINI REVIEW

Micro-organism–gold interactions

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This review examines interactions between micro-organisms and gold with emphasis on gold binding to bacteria, algae, yeasts and fungi. Aspects of gold toxicity and resistance in micro-organisms are also reviewed.

Keywords: biosorption, metals bioremediation, metal-microbe interactions, precious metals

Introduction

Interactions of microorganisms with numerous metals such as copper, silver, nickel, zinc, cadmium, mercury, lead, including sodium, calcium, magnesium, manganese and iron have been well studied and documented (Hughes & Poole 1989, Brierley 1990, Gadd 1990, Beveridge *et al.* 1997). Furthermore, pollution of the environment by toxic metals and radionuclides has long been a priority concern for environmental protection. Recently, increased attention has also focussed on the biotechnological potential of micro-organisms for removal of toxic metals from waste effluents and industrial process streams (Gadd & White 1993).

The removal of metals, radionuclides or metalloid species from solution by biological material, particularly by physico-chemical interactions such as adsorption or ion exchange is termed biosorption (Volesky 1990). Bacteria have been used in numerous physiological and genetic studies of metal accumulation, resistance and detoxification (Trevors *et al.* 1986, Beveridge 1989a). Fungi and yeasts have received considerable attention in connection with metal biosorption, particularly because waste fungal biomass (for example *Aspergillus niger* a major waste product of citric acid biosynthesis) is available as a by-product from several industrial fermentations (Gadd 1990).

Numerous algal strains have been used as biomonitors of metal pollution in lakes, rivers and oceans (Wong *et al.* 1982, Trevors *et al.* 1986), and for heavy metal ion recovery from aqueous solutions (Gale 1986, Gadd 1988, Kuyucak & Volesky 1990).

Micro-organisms have played important roles in solubilizing metals and in mineral deposition in the natural environment. These processes can be exploited in the recovery of metals and in the *in situ* decontamination of metal-polluted waste sites.

Most current research interests, however, center on the recovery of high value metals. Fluctuating prices of gold and high demand for the metal by the industry make it necessary to process lower grade ores, waste-rock dump materials and scrap residues. Biosorption has been suggested as an economical alternative to the existing metal recovery technologies. This paper reviews interactions of gold with micro-organisms, a high value, biologically non-essential element widely used for jewelry and medicinal purposes.

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Chemistry of gold

Gold is a soft yellow metal with the highest ductility and malleability of any element. Native gold is one of the most stable elements. However, despite its surprising stability, gold is extensively scattered in nature and pathways of its biogeocycling remain largely unexplored. Native gold contains about 40 elements as impurities, of which silver and copper are the most common. Gold is chemically unreactive, and is not attacked by oxygen or sulfur-containing ligands under alkaline conditions. Compounds and complexes of gold with ammonia, amines and cyanide occur (Cotton & Wilkinson 1980).

According to Pearson (1963) Au^{+} and Au^{3+} ions are classified as class b or soft metal ions. Biologically important metals and ligands are hard or borderline hard. Fundamental cellular constituents such as polysaccharides and proteins and potential binding groups such as hydroxyl, phosphate and carboxyl are generally hard ligands, usually with O as the donor atoms. It must be emphasized that some biological ligands are soft, notably the sulfur-containing side chains of cysteine and methionine residues. In contrast to the hardness of living systems, the most commonly studied toxic metals associated with environmental pollution are soft, such as Cd^{2+}, Co^{2+}, Hg^{2+}, Pb^{2+}, Cu^{2+} and Ag^{+}. Complexes of Au^{+} and Au^{3+} are both soft metal ions and any ligand interactions are likely to occur with sulfur- or nitrogen-donor ligands. Au^{+} ion, has high affinity for soft ligands and forms predominantly complexes of linear two-coordination $(CN - Au - CN)^{-}$, $(Cl - Au - Cl)^{-}$, Au^{3+} ion, forms four-coordinate square planar complexes such as $AuCl_4^{-}$, AuI_4^{-}, that are often strong oxidizing agents, and of little medical or environmental interest.

Various Au(I) thiols are biologically active and the sodium gold thiomalate (myochrisine) and thioglucose derivatives (water soluble) have been used as anti-inflammatory drugs in the treatment of rheumatoid arthritis (Sadler 1976). Certain complexes of Au(I) have been found to have antimicrobial and antifungal activity in a defined medium (Berners-Price *et al.* 1988). Gold has also been used for labeling enzymes and proteins for X-ray diffraction study, since it can readily be located. The usual labeling agents are $Au(CN)_2^{-}$, $Au(Cl)_4^{-}$ and AuI_4^{-} (Verschueren *et al.* 1993). Reviews on the chemistry of gold compounds in aqueous and biological systems appear elsewhere (Puddephatt 1978, Korobushkina *et al.* 1983).

Gold binding to bacteria and cyanobacteria

Relatively little research has been completed on interactions of gold with bacteria. Most of the research has been centered on studies of gold uptake and accumulation by *Bacillus*, *Spirulina* and *Pseudomonas* species (Beveridge & Murray 1976, Higham *et al.* 1986, Gee & Dudeney 1988, Karamushka *et al.* 1991d, Garbara *et al.* 1992, Ulberg *et al.* 1992, Southam & Beveridge 1994, Savvaidis, unpublished results).

Pseudomonas cepacia has been shown to produce a yellow low-molecular weight protein (thiorin) during growth in medium containing gold compounds. The presence of gold in the protein could not, however, be demonstrated (Higham *et al.* 1986). The most efficient use of such proteins would be to maximize their production during growth, isolate the protein product and test it for subsequent metal sequestration.

The interaction of *Bacillus* species with gold in ionic and colloidal state has been investigated (Ulberg *et al.* 1986, Karamushka *et al.* 1987a,b). The mechanism of colloidal gold fixation by *Bacillus* cells was dependent on the cell surface and involved a number of functional groups (provided by proteins and carbohydrates), usually present within the anionic cell surface. The mechanism of gold binding to the cells may depend on the strain of microorganism, and type of gold (ionic or colloidal) being used (Garbara *et al.* 1989).

The binding of $Au(Cl_4)^{-}$ to the cell walls of *Bacillus subtilis* was examined by Beveridge & Murray (1976). They suggested that the nature of interaction involved the reduction of tetrachloroaurate(III) to Au(0) on the cell surface. It was also noted that reduction of gold was unique since other bound metals, including Ag^{+} were not reduced. It was also suggested that cell wall reactive sites acted as nucleation sites for gold accumulation with the formation of microscopic elemental gold crystals. The accumulation of ionic and colloidal gold by *Bacillus* species was investigated by Ulberg *et al.* (1986), and Karamushka *et al.* (1987a,b). They found that gold accumulation was dependent on the fine features of the chemical structure of the cell envelopes, and involved functional groups of proteins and carbohydrates. In other studies of interactions involving colloidal or ionic gold with bacteria *Bacillus* spp., heterocoagulation of *Bacillus* cells with gold was noted, and the accumulation of gold was found to be directly dependent on the metabolic activity of the cell culture (Karamushka *et al.* 1990b, c, Ulberg *et al.* 1992). It was also shown that gold accumulation

depended on the metabolic reactions proceeding on the plasma membrane. In particular, hydrolysis of ATP by ATPase coincided with efficiency of plasma membrane vesicles to bind gold from the medium. The use of metabolic inhibitors showed that gold concentration depended on proper functioning ATPase. At low gold concentrations (0.030 mM) in the medium (gold as tetrachloroaurate), the *Bacillus* cells (*Bacillus cereus* B-4368, *Bacillus subtilis* B-1727) lost the ability to concentrate gold (ionic or colloidal) in the presence of metabolic inhibitors including, dinitrophenol, pentachlorophenol and sodium azide (Karamushka *et al.* 1990b,c).

These researchers suggested that gold accumulation by the *Bacillus* cells proceeded by an initial, reversible phase, followed by an irreversible phase. During the first phase, cells can release the localized (accumulated) gold back in the growth medium under the influence of inhibitors, whereas in the second irreversible phase the inhibitors exerted no such effect (Karamushka *et al.* 1992).

Energy-dependent concentration of Au^{3+} by the cyanobacterium *Spirulina platensis* was also reported (Karamushka *et al.* 1995). Accumulation of Au^{3+} by living *Spirulina* cells increased as the pH increased (pH 3–8), whereas for inactivated cells, amount of gold was maximum at pH 3.0 and decreased as the pH increased (pH 4–8). The process of gold accumulation by the cells was also inhibited by the metabolic inhibitors sodium azide (0.1 mM) and dicyclohexylcarbodiimide (0.01 mM). It was concluded that accumulation of Au^{3+} by *S. platensis* cells is a complex process consisting of a passive binding of the metal by the cellular structures and its energy-dependent localization in the cell.

The interactions of gold(III) chloride with *Bacillus subtilis*, and the cyanobacterium *Spirulina platensis* were characterized by Gee & Dudeney (1988). Gold was shown to be selectively adsorbed from simulated leachate solutions containing Au^{3+}, Cu^{2+}, Fe^{2+} and Zn^{2+}, with a resulting increase of at least an order of magnitude in the gold concentration in the cells. Kinetic studies showed gold adsorption by the cells was rapid, and involves two types of binding sites.

A filamentous marine cyanobacterium *Plectonema terebrans* accumulated Au^{3+} in its sheath (glycocalyx) possibly by adsorption from a solution containing $AuCl_3 \cdot H_2O$. The possibility of gold accumulation in the cells was not ruled out (Dyer *et al.* 1994).

The recovery of gold from gold-thiourea solutions using various types of microbial biomass, including *Spirulina platensis* and *Streptomyces erythraeus* was investigated (Savvaidis, unpublished results). The binding of gold to *S. platensis* was rapid and complete within 5 min from contact time. The binding of gold to *S. platensis* cells was independent of pH (2–7), suggesting that covalent bond may have formed between gold and the cells, as also reported by other researchers (Darnall *et al.* 1986). In all other cases, the binding of gold to the cells was pH-dependent suggesting electrostatic interaction between the gold and the cells. *S. platensis* cells had the highest affinity ($K_d = 2.54\ mM^{-1}$) for gold from gold-thiourea solutions, and also the highest gold capacity for gold (3.3 mg gold per g biomass) even at low pH values (2–5), compared to other types of waste biomass tested. In all cases, binding of gold appears to involve one type of binding site, as shown by the linearity of Langmuir plots. It was speculated that the mechanism of gold accumulation by cells could initially involve adsorption of gold (or gold-thiourea complex) by cells with binding of gold also inside the cells (Savvaidis, unpublished results).

Gold binding to algae and microalgae

Whether viable or nonviable, algal cells have a remarkable affinity for gold ions. Algal mats near the coast of Sri Lanka can accumulate gold up to 1.1 p.p.m. (Dissanayake & Kritsotakis 1984). Most of what is known about gold binding to algae has been determined from studies on the freshwater species of *Chlorella* (Greene *et al.* 1986, Hosea *et al.* 1986, Watkins *et al.* 1987, Karamushka *et al.* 1990a). However, various algal and microalgal species have been tested for their potential to recover gold from aqueous solutions (Darnall *et al.* 1988, Kuyucak & Volesky 1990, Karamushka *et al.* 1991c, Wilde & Benemann 1993).

The interactions of Au^{+} and Au^{3+} complexes with *Chlorella vulgaris* have been studied by Greene *et al.* (1986). The algal cells accumulated both Au^{+} and Au^{3+} from aqueous solutions with high affinity. The extent of gold adsorption was dependent on the presence of competing ligands in the solution. Tetrachloroaurate(III) and Au(I) sodium thiomalate were rapidly adsorbed by the cells over a wide pH range, whereas dicyanoaurate bound more slowly and in a highly pH-dependent manner, with maximum binding obtained near pH 3.0. Experiments suggested that the mechanism of tetrachloroaurate(III) interaction with *Chlorella vulgaris* involved rapid reduction of Au^{3+} to Au^{+}, followed by a slow reduction to Au(0). The accumulation of elemental gold by lyophilized preparations of the alga *Chlorella vulgaris* was investigated by Hosea *et al.* (1986). Gold was bound to the algae upon suspending dried algal

cells in solutions containing hydrogen tetrachloroaurate(III). The amounts of ionic and atomic algal-bound gold were determined by thiourea extraction and it was found that the amount of algal-bound atomic gold produced from ionic gold increased with time. It was suggested that at least three classes of gold-binding sites were present on the algal cell. One class is composed of weak-binding sites, provides an environment which permits the reduction of bound Au^+ to Au(0). A second class associated with stronger binding does not permit Au^+ reduction. The third class, presumably of intermediate strength, does permit gold reduction, but only after elemental gold has accumulated elsewhere on the algal cell. The effect of Au(0) on the binding ability of gold-bound algae was also examined and an apparent enhancement (22%) of gold-binding ability was reported. One possible explanation was that the gold may migrate from the binding site to a growing gold crystal during or after reduction, freeing the binding site for additional gold binding. It was also suggested that gold atoms deposited on the algal cell during reduction of bound Au^+ serve as nucleation sites, and additional gold is deposited directly into a growing crystal without first binding to the algae. Either of these mechanisms would be consistent with the suggestion of Beveridge & Murray (1976) that Au^{3+} bound on the cell walls of *Bacillus subtilis* initiates a seeding process which results in the formation of elemental gold.

Polyacrylamide-immobilized *Chlorella vulgaris* was used to selectively recover a number of heavy metal ions from solutions, including Zn^{2+}, Cu^{2+}, Hg^{2+} and Au^{3+}, by means of pH variation (Darnall *et al.* 1986). Depending on how binding to the alga was affected by pH, most of the metal ions tested were divided into three categories. Group one metal ions that bound to *Chlorella vulgaris* rather independently of pH values between 2.0 and 7.0. These included Ag^+, Hg^{2+}, Pd^{2+}, $Au(Cl)_4^-$, Au^{3+} and gold(I) thiomalate. Gold, silver and palladium are classified as soft according to Pearson (1963). These metal ions will form covalent bonds with soft ligands such as amine and sulfydryl groups. The binding interactions between soft (polarizable) metal ions and soft ligands, generally, are affected minimally by ionic interactions and pH in contrast to interactions between hard metal ions and hard ligands. Group two metal ions bound more strongly to the cells as the pH increased from 2.0 to 5.0. This group consisted of those ions intermediate between soft and hard metal cations, including, Cu^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, and the hard metal ions Be^{2+}, and Al^{3+}. Group three metal ions bound more strongly to *Chlorella vulgaris* at pH 2.0 than at 5.0. They included the oxoanions MoO_4^{2-}, SeO_4^{2-} and the anionic complexes $Au(CN)_2^-$ and $Pt(Cl)_4^{2-}$. At low pH values these metal ions were bound to the cells by electrostatic interaction with positively charged groups, such as amines or imadazoles. X-ray absorption spectroscopy was used to investigate the binding of *Chlorella vulgaris* to Au^+ and Au^{3+} complexes by Watkins *et al.* (1987). These techniques, namely X-ray absorption near-edge structure (XANES) and the extended X-ray absorption fine structure (EXAFS) indicated that the predominant oxidation state of the gold in the algal complexes is Au^+. It was suggested the mechanism of gold binding (sodium gold(I) thiomalate) to the cells involved ligand-exchange reactions leading to the formation of bonds between Au^+ and sulfur or nitrogen donor atoms contained in the algae. There is some evidence that binding of Au^{3+} to cells most likely involved nitrogen donor atoms.

An algal biosorbent used to recover gold from dilute aqueous solutions and an extremely high capacity for gold (420 mg gold per g biomass) was obtained (Kuyucak & Volesky 1988, 1989a,b,c). The mechanism of gold deposition was studied by chemical and instrumental analysis. X-ray diffraction along with ESCA (X-ray photo electron spectroscopy) indicated that the gold taken up by the biosorbent was deposited in its elemental form. Infra-red spectra showed that carbonyl (C=O) groups on the biomass were involved in the binding of gold. Electron microscopy of the gold-laden biomass revealed that the cell wall of the algal biosorbent was the principal location for gold deposition initially, whereas the metal penetrated into the cell when contact time was increased.

The binding of tetrachloroaurate(III) to several algal species, including *Rhodymenia palmata*, *Porphyra yezoensis*, *Laminaria japonica*, *Eisenia bicyclis*, *Macrocystis pyrifera*, *Cyanidium caldarium* and *Chlorella vulgaris* was examined by Darnall *et al.* (1988). All algal species tested were found to adsorb tetrachloroaurate(III), although the kinetics, pH dependence and binding capacities differed amongst certain species. Amongst all algal species examined, *Chlorella vulgaris* cells rapidly reduced tetrachloaurate(III) to Au^+, and then slowly reduced Au^+ to Au(0). This evidence for gold reduction supports the view that algae may have played a significant role in the transport and deposition of gold in the environment.

The screening of various algal species for gold binding is important in understanding the transport mechanisms of gold in the environment and in the

development of methods for the recovery of gold from solutions. Various algal species including green algae, red algae and blue-green algae, were screened for their ability to accumulate Au^{3+} from solutions (Karamushka *et al.* 1991c). Energy-dependent accumulation of gold was exhibited by cells of most species. The accumulation of Au^{3+} by cells was inhibited by metabolic inhibitors that affected ATP synthesis, whereas ATPase appeared to be essential for Au^{3+} accumulation. In an effort to understand the mechanism of gold accumulation, the effect of pH and of metabolic inhibitors on the recovery of tetrachloroaurate salts by *Chlorella vulgaris* was investigated (Karamushka *et al.* 1991a,b). Incubation of Au^{3+} cells resulted in the binding of gold after 30 min of contact time, whereas heat-inactivated cells accumulated less Au^{3+}. Energy-dependent Au^{3+} accumulation was most intensive at alkaline pH, decreased in the dark, and was inhibited in the presence of over 1 μM arsenate, over 0.01 mM fluoride, 1 mM azide, 10 μM DCCD and 0.1 mM 2,4-dinitrophenol. In the dark, Au^{3+} accumulation was stimulated by addition of ATP which also neutralized the effect of sodium azide but not that of dinitrophenol. Energy-dependent Au^{3+} accumulation was also observed for other species tested.

Immobilized non-viable algal preparations have received detailed attention in exploring a new technology in bioremediation of precious metals such as gold (Bedell & Darnall 1990). Fluidized beds of alginate and polyacrylamide-immobilized algae, for example of *Chlorella vulgaris* and of the cyanobacterium *Spirulina platensis*, have been used to remove a variety of metals, including Cu^{2+}, Pb^{2+}, Zn^{2+} and Au^{3+} from mixtures of metal solutions, and several systems for selective metal recovery have been devised (Darnall *et al.* 1988, Green and Darnall 1990). A commercial algal preparation currently being used for metal recovery is AlgaSORB (Bio-Recovery Systems Inc., Las Cruces, NM, US). This proprietary material contains algal cells immobilized in a silica matrix and is used in batch and column systems. Columns are slurry-packed with immobilized algal particles, 40 to 100 mesh size, and used for metal biosorption. AlgaSORB has been successfully used for recovery of various metal ions, including Au^{+}, and Au^{3+}, from industrial effluents and process streams (Bedell & Darnell 1990). *Chlorella homosphaera* cells immobilized on sodium alginate were used to treat solutions containing cadmium, zinc and gold. Metal adsorption by the cells was complete within 60 min for zinc and cadmium, and 30 min for gold (Costa & Leite 1991).

Gold binding to yeasts, fungi and other metabolic products of micro-organisms

Relatively little work has been done on the interactions of gold with yeasts and fungi. Features of the fine structure of cells of the yeast *Candida utilis* YKM-1668 were examined using electron microscopy, when cells were grown in a synthetic medium containing metallic gold or ionic Au^{3+} (Biryuzova *et al.* 1987). Gold was adsorbed by the cells and was deposited in almost all organelles except mitochondria. Incubation of gold(III) solution and of *Candida* cells in a gold(III) solution for 6 h resulted in the deposition of gold within the cell wall and periplasm. Metallic (powdered) gold on the other hand, was not adsorbed rapidly by the cells due to the high Au(0) concentration used (200 mg/l). Despite the slower adsorption of Au(0) by cells, after 6 h of incubation, gold was found within the cell wall, in the periplasm, in the cisternae of the endoplasmic reticulum and the membranes as shown by light and electron microscopy. The slower accumulation of powdered gold by yeast cells in comparison with the ionic Au^{3+} was attributed to the inability of cells to convert Au(0) into the soluble ionic form. The interactions of gold(III) chloride and the fungus *Aspergillus niger*, as well as other types of micro-organisms (*Spirulina platensis* and *Chlorella vulgaris*), were characterized by Gee & Dudeney (1988). Gold was shown to be selectively adsorbed at high efficiency from simulated leach solutions, containing Au^{3+}, Cu^{2+}, Fe^{2+} and Zn^{2+}. Adsorption of gold by various cell types examined was rapid and kinetic studies indicated that the process of gold biosorption involved two types of binding sites. The uptake of gold from gold–thiourea solutions using waste biomass, (*Saccharomyces cerevisiae*) was also investigated (Savvaidis, unpublished results). The uptake of gold was rapid (complete within 2 min of contact time) and the process was dependent upon pH (values 4–6), suggesting electrostatic interactions may be involved in the binding between the gold ions (or the gold–thiourea complex) and the negatively charged groups on the cell surface of the yeast.

Yeast Cu-metallothionein (Cu-MT) genes has the potential in metal recovery since it can bind metals other than Cu, for example, Cd, Zn, Ag, Co, and Au, although these metals do not induce yeast metallothionein production (Butt & Ecker 1987). Consequently, engineering of yeast strains for constitutive expression of metallothionein genes may be one approach to overcome this problem (Butt & Ecker 1987).

Pseudomonas cepacia has been shown to produce a yellow low-molecular weight protein (thiorin) during growth in medium containing gold compounds. The presence of gold in the protein could not, however, be demonstrated (Higham *et al.* 1986). The most efficient use of such proteins would be to maximize their production during growth, isolate the protein product and test it for subsequent metal sequestration.

It has, however, been shown that gold is strongly complexed by metallothioneins (Laib *et al.* 1985). From studies of interaction of Au^{+} and Au^{3+} complexes with biological molecules *in vitro*, it was shown that the predominant oxidation state of gold is +1, that Au^{3+} is reduced to Au^{+} by several ligands (i.e. cysteine), and that under certain conditions, further reduction of Au^{+} to Au(0) may occur (Sadler 1976). In another study, amino acids produced by bacteria were found to solubilize native gold at neutral to alkaline pH (Korobushkina *et al.* 1974, 1976, Mineyef, 1976). Examination of the infrastructure-red spectrum of the auriferous amino acid fraction showed that gold-amino acid complex formation involved the nitrogen donor atom of the amino group (Korobushkina *et al.* 1976). In acidic pH, the interaction of Au^{3+} and amino acids resulted in a slow reduction of gold to a metallic state. In alkaline pH, certain amino acids, namely, glycine, alanine, valine and phenylalanine formed complexes with gold where the amino acid coordination by the metal is effected through the amino and carboxyl groups. The stability of gold(I)-amino acid complex varied with their redox potentials (Korobushkina *et al.* 1983). Of the metabolic products of micro-organisms, amino acids play the primary role in gold dissolution. Peptides, proteins and nucleic acids also dissolve gold. A glycoprotein has been implicated as a possible site for gold binding (Ulberg *et al.* 1986). In this case, the metal was presented as a colloidal gold obtained by reduction of chloroauric acid, with the gold-microbial interaction visualized as a coagulative flocculation. Whole cells and butanol extracts of outer membranes of a *Bacillus* species and a *Pseudomonas* species gave the flocculative response. A distinctive band was observed in outer membrane extract, termed 'gold binding factor', using sodium dodecyl sulfate gel electrophoresis (Ulberg *et al.* 1986).

Gold toxicity and resistance in micro-organisms

Metal toxicity to man is an increasingly important environmental concern. Most metal ions, when present at sufficient concentrations, have the potential to be toxic to biological systems. While there are reports in the literature of studies on metal toxicity and resistance to micro-organisms (Hughes & Poole 1989, Silver & Phung 1996), none of these deal with gold.

Heavy metal resistance has been well studied for metals such as Cd and Hg, and also for the essential metal Cu (Silver & Phung 1996). Metal resistance of micro-organisms is frequently plasmid encoded. Bacterial plasmids encoding resistance determinants for toxic metal ions have been reported for metals including, Hg^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}, Sb^{2+}, Tl^{+}, Zn^{2+}, Cu^{2+}, AsO_2^{-}, AsO_4^{3-}, Co^{2+}, CrO_4^{2-} TeO_3^{2-} and Ag^{+} (Silver & Phung 1996). Not much information is available on bacterial resistance to precious metals, except for Ag^{+}, where preliminary studies indicate that reduction of Ag^{+} to Ag(0) is not the mechanism of resistance. Silver efflux has not been demonstrated as the basis for this resistance (Slawson *et al.* 1990, Silver & Phung 1996).

Reduction of Au^{3+} to Au^{+} and finally to Au(0) by algal cells has been reported. It is not known if this reduction could be related to the mechanism of resistance. Gold resistant bacteria, however, have been reported (Higham *et al.* 1986). A strain of *Pseudomonas cepacia* was isolated from natural water and adapted to grow in a defined liquid culture medium containing millimolar concentrations of gold(I) thiolates, including the antiarthritic drug gold(I) thiomalate. The cells became very large, accumulated polyhydroxybutyrate and gold, and excreted a yellow protein (thiorin) into the culture medium. Thiorin was shown by ^{1}H-NMR, amino acid analysis and gel filtration chromatography to be of low molecular weight. This effect was not observed with the gold(III) complexes tested, which were reduced to metallic gold in the medium (Higham *et al.* 1986). It was suggested that gold may inhibit one of the depolymerase enzymes in the breakdown of polyhydroxybutyric acid, or alternatively interfere with nitrogen metabolism of *Pseudomonas cepacia.* In another study, gold resistant strains, including fungi and heterotrophic bacteria, were isolated from natural waters polluted with heavy metals. All strains were able to accumulated gold, along with other metals such as silver, nickel and cadmium from dilute metal solutions (5 mg l^{-1}) (Wnorowski 1991).

Toxicity of gold to micro-organisms may vary depending on the concentration and type of gold ion, the presence of competing metal ions in solution, the pH of the solution and the composition of growth medium. The presence of trace quantities of Au^{3+} or Au(0) in the growth medium may not be

toxic for the cell and thus the gold ion may be deposited in certain parts of the cell wall and the periplasm. Such deposition of gold was noted in the yeast *Candida utilis* VKMY-1668, when cells were grown in a synthetic medium containing Au^{3+} and Au(0). Gold was deposited in almost all organelles except the mitochondria, and it was suggested that gold probably blocked processes associated with growth rather than substrate uptake (Biyuzova *et al.* 1987). Mechanisms of gold resistance in both prokaryotes and eukaryotes remain largely unknown to date. Exposure to gold, however, may induce cell adaptation and cell resistance as in the case of gold chloride, sodium aurothiomalate and auranofin. Auranofin induces increased synthesis of metallothionein which may have metal-binding and homeostatic properties. Experimental evidence suggests cellular adaptation as a potential mechanism for gold resistance (Wollheim 1988).

Limited information is available on studies of gold toxicity and resistance in micro-organisms and thus there is need for further studies on gold interactions and micro-organisms in order to gain insight in these mechanisms. From the biotechnological point of view, it is useful to have a gold resistant strain that could be used to accumulate high levels of gold and survive under harsh conditions used in gold recovery processes of sulfide ore minerals. Yet, the fundamental mechanism of gold resistance in micro-organisms is not known or understood to date, and the next few years should see increased progress and effort in studying and understanding gold interactions and micro-organisms especially at the molecular level.

Gold geomicrobiology

Gold occurs widely in the biosphere and in very minor amounts in various rocks forming the upper lithosphere. It occurs at high concentrations in deposits enriched with organic matter. Soil in gold-bearing areas generally has high concentrations of gold as organo-mineral complexes. Water in rivers may contain occasionally up to 1 g of gold per 1000 l water. Gold content in marine water is two to three orders of magnitude lower than in the lithosphere. The presence of gold in the biosphere thus suggests that it is involved in cycling processes. Prokaryotes have existed for at least 3.6 billion years and it is reasonable to assume they have contributed, over the course of time, to the initial phases of mineral development (Beveridge 1989b). It is generally understood that microbial and biochemical processes may directly or indirectly play a role in the formation of a great variety of mineral resources, including the noble metals (Korobushkina *et al.* 1983, Mann 1992, Dyer *et al.* 1994).

For many years micro-organisms have been implicated in weathering processes, leaching and deposition of mineral ores. Metal deposition of micro-organisms is of great importance in biogeochemical cycles, for example microfossil and mineral formation, iron and manganese deposition and uranium and silver mineralization (Beveridge 1989b). The concentration of gold in the stream beds of various regions around the world is still a long-standing issue.

The formation of certain gold deposits in South Africa was attributed to the involvement of Precambrian algal blooms and bacteria (Zumberge *et al.* 1978). Preliminary evidence of gold biomineralization by *Pedomicrobium*-like budding bacteria, known also to be involved in iron and manganese oxide deposition processes was provided (Watterson 1991). It is generally considered that bacterial mineralization is the result of the interaction of metallic ions with bacteria in natural environments. Even in acid lake environments, whose low pH levels should diminish heavy metal precipitation, bacteria were still capable of promoting mineralization (Ferris *et al.* 1989, Southam & Beveridge 1992). Gold usually exists in solution in the form of Au^{+} of Au^{3+} complexes, which have been shown to be easily reduced at the cell surface, and thus induce metallic gold deposition (Darnall *et al.* 1988). A marine cyanobacterium, *Plectonema terebrans*, accumulated gold from solution in its sheath (Dyer *et al.* 1994). It was suggested that the sheath (glycocalyx) of cyanobacteria, (only part of the cell to be preserved as fossil record), gold-coated *Pedomicrobia* and gold-replaced setae, all supplied key evidence of bacteriform gold. A laboratory simulation was developed to provide mechanistic information about placer (nugget) gold development in the natural environment. In this model, ionic gold was accumulated by *Bacillus* species 168 (116.2 μg mg^{-1} dry weight) as fine-grained intracellular colloids (5–50 nm). During the low-temperature diagenesis experiment (60 °C), the release of organics due to bacterial autolysis coincided with the *in vitro* formation of hexagonal-octahedral gold crystals (20 μm). In addition to achieving a fundamental understanding into secondary gold deposition a significant economic benefit could be realized by employing this microbially mediated environmentally safe method to concentrate gold in fine-grained placer deposits that are presently not worth mining (Southam & Beveridge 1994).

It has been found that micro-organisms and their metabolic products are involved in the dissolution and precipitation of gold in the natural environment. Such metabolic products of micro-organisms may include amino acids produced by bacteria (Korobushkina *et al.* 1974, 1983, Mineyev 1976), as well as humic and fulvic acids (Baker 1978, Bergeron & Harrison 1989) involved in transport of gold. The process of gold dissolution and precipitation depends on pH and redox conditions of the medium, and the presence of oxidants, which can change native gold into an ionic form. Changes of pH and redox potential, which may be caused by bacterial activity, may lead to the reduction of gold into a metallic state and its precipitation. Considering the global aspects of gold redistribution in the biosphere, it is certain that gold migration is aided by the interaction of gold with organic compounds with the final dissolution and precipitation of gold.

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