EVIDENCE FOR A MICROBIALLY MEDIATED BIOGEOCHEMICAL CYCLE OF GOLD – A LITERATURE REVIEW

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INTERACTIONS OF BIOTA WITH GOLD – AN INTRODUCTION

Gold is widely distributed in the biosphere. Various organisms have displayed the ability to accumulate gold (Korobushkina *et al.* 1983). Sea urchins, sea stars and crabs have been shown to accumulate gold (Korobushkina *et al.* 1983). Some land plants have been shown to accumulate gold, such as the thiocyanate containing carrot family (*Umbelliferae*) (Mossman *et al.* 1999) and the monocotyledon *Typha* (Boyle 1979); in the marsh horsetail (*equisetum pallustre*) from Oslany in the Czech Republic up to 610 ppm of gold were detected (soil background was measured at 0.2 ppm) (Babicka 1943); in samples of the bluebush (*Maireana*) elevated gold concentrations were detected and its use as an indicator for underlying deposits in geochemical exploration in Australia has been proposed (Lintern *et al.* 1997); *Eucalyptus spp.* bark samples from the Ballart gold field in Victoria, Australia, contained up to 150 ppb of gold (Arne *et al.* 1999); several fungal species from the genus *Penicillium* were also shown to accumulate gold (Boyle 1979).

Phylogenetically, ecologically and metabolically prokaryotes, i.e. bacteria and archaea, are the most versatile and adaptable organisms on earth and have long been thought to influence gold solubilization, mobility and precipitation. Since the 1960s researchers have conducted experiments with bacteria and studied the effects of auric, colloidal and native gold on various species. After the discovery of the effect of *Thiobacilli* on sulfide minerals in 1947, various studies were and are being conducted on the effect of iron- and sulfur-oxidising bacteria on gold bearing metal sulfides; gold was shown to be liberated in the process of microbial sulfide decomposition (e.g. Langhans *et al.* 1995). Watterson (1992) discovered microbial fossils or pseudomorphs, which he described based on morphological features as *Pedomicrobium* spp., found on gold flakes and nuggets from placer deposits in the USA. Other authors have reported similar findings from nuggets from Australia, Venezuela and South Africa (Hallbauer & Barton 1987, Bischoff 1994, 1997, Bischoff *et al.* 1992). Recent evidence indicates, that the formation of the Witwatersrand deposit in South Africa, which is the largest known paleo-placer deposit, might be microbially mediated (Mossman *et al.* 1999).

Two questions concerning the involvement of bacteria with gold have to be addressed: how do prokaryotes manage to accumulate gold and why do they do it? Recent studies on the biochemistry of metal accumulation have answered the first question. The answer to the second question remains speculative. Since gold is generally believed to be a non-essential trace element for microbial nutrition. However, Babicka suggested (1943) that low gold concentrations might stimulate the activity of certain plants and microorganisms. Gold has been discovered to play a functional role in an enzyme *Microcosus luteus*, a common air-borne bacterium, which is used in the oxidation of methane (Levchenko *et al.* 2002). However, it is likely that gold is a constituent of other enzymes in other species, which have not yet been cultured. Au^{3+} is toxic to microorganisms in high concentrations and microorganisms might be able be able to reduce gold as a way to detoxify environment (Mossman 1999, Karthikeyan & Beveridge 2002).

MICROBIAL INFLUENCES ON THE SOLUBILISATION OF GOLD

Several studies have been undertaken to evaluate the influence of bacteria on the solubilization and mobility of gold. Pares and Martinet (1964) (in Boyle 1979) found that gold from laterites and various other gold bearing materials could be solubilized by autotrophic bacteria, such as nitrogen-fixing species, and to a smaller extent by various heterotrophic bacteria, such as *Bacillus subtilis*, *Serratia marcescens*, and *Pseudomonas fluorescens*, which had been isolated from soil, water and air. Lyalikova and Mokeicheva (1969), who assessed the role of bacteria in the migration of gold during the oxidation of deposits, found that numerous heterotrophic bacteria were able to dissolve gold. In other experiments they found that various sulfur components, which accumulated in solution after the oxidation of sulfide minerals by sulfur bacteria, contributed to the gold dissolution. A strain similar to *Bacillus alvei* isolated from a gold deposit dissolved up to 600 $\mu g\ L^{-1}$ in 3 weeks (Boyle 1979). Strains of *Bacillus megaterium*, *Bacillus mesentericus*, *Pseudomonas liquefaciens*, and *Bacterium nitrificans*, isolated from gold bearing deposits were found to dissolve up to 35 $mg\ L^{-1}$ of gold during 30 days of incubation (Korobushkina 1974). Numerous studies have been undertaken to date to assess the ability of iron- and sulfur oxidising bacteria and archaea, such as a strain of *Thiobacilli*, *Leptospirilli*, or the extreme thermophiles *Sulfolobus, Acidianus and Metallosphaera*, to dissolve gold (e.g.,

Iglesias & Carranza 1995, Sandstroem & Peterson 1997, Ubaldini *et al.* 2000). These experiments were generally successful and several times more gold was dissolved by these strains compared to sterile controls. Today some of these strains are used in industrial bioleaching processes to extract metals from natural ores and industrial residues (Bosecker, 1997).

Apparently, different processes to dissolve gold are used by autotrophic iron- and sulfur-oxidising- and heterotrophic bacteria. Iron- and sulfur-oxidizing bacteria mediate the dissolution of gold while oxidizing the metal sulfides, whereas heterotrophs dissolve gold apparently by excreting corrosive organic acids. During the process of microbially mediated metal sulfide-oxidation gold is liberated from the pyrite or arsenopyrite, where it existed as a lattice component in free-formed blebs. The oxidation of metal sulfides also creates thiosulfate, which appears to form stable complexes with gold:

$$Au + 0.5\ O_2 + H_2O + 2S_2O_3^{2-} \lozenge Au(S_2O_3)_2^{3-} + OH^-$$

The gold-thiosulfate-complex is stable under a wide range of Eh- (-0.17 to 0.76) and pH-conditions (5 to 10).

Gold dissolution by heterotrophic bacteria, which are the dominant group in most soils (Paul & Clark 1996), appears to be mediated by organic products of the microbial metabolism (Korobushkina *et al.* 1983). Gold is dissolved by organic acids, such as amino acids, which are produced and excreted by bacteria during their metabolism. Bacteria isolated from auriferous deposits have been shown to release aspartic and glutamic acids in high quantities to the environment (Savvaidis *et al.* 1997). In addition other organic metabolites such as nucleic, pyruvic, lactic, oxalic, formic and acetic acid are excreted into the environment (Kuesel *et al.* 1999, Reith *et al.* 2002). Some of these organic metabolites have been shown to be able to form stable complexes with gold (Vlassopoulos *et al.* 1990). An examination of infrared spectra of auriferous amino acid fraction showed that gold-amino acid complex formation involved nitrogen from the amino group (Korobushkina *et al.* 1976). The stability of these gold-amino-acid- complexes varies with their redox-potentials and have been ranked accordingly as follows (Korobushkina 1983): cysteine > histidine > asparagine > methionine > glycine, alanine, phenylalanine. Another metabolite some bacteria use in the dissolution of gold has recently been assessed (Campbell *et al.* 2001). *Chromobacterium violaceum* is a cyanogenic (cyanide-producing) organism. Cyanide is known to form stable complexes with gold (Gray 1998). Experiments have shown that when *Chromobacterium violaceum* is present in a nutrient broth it formed a biofilm and could complex and solubilize 100 % of the gold on glass test slides within 4 to 7 days (Campbell *et al.* 2001).

MICROBIALLY INDUCED PRECIPITATION OF GOLD

The ability to bind metals is an inherent characteristic of many microorgansims, such as bacteria, archaea, algae or fungi (Beveridge *et al.* 1983), and has played an important role in the formation of various geologic deposits (Trudinger 1976). Generally, microorganisms can enrich elements such as gold in two ways by adsorption, i.e. deposition along the cell wall, and by absorption, i.e., enrichment within the cell. Both processes appear to play an important role in the precipitation of gold by microorganisms, and thus in the biomineralisation of gold, which as recent evidence suggests let to the formation of some of the world largest gold deposits, such as the Witwatersrand paleo-placer deposit in South Africa (Mossman *et al.* 1999). Gold in the Witwatersrand deposits and in deposits of deeply weathered Archaean metamorphic rock from Western Australia is present as secondary octahedral gold particles (Wilson 1984, Frimmel *et al.* 1993, Minter *et al.* 1993). The geochemical, abiotic formation of these particles through mobilisation of the original detrital, dispersed gold involves temperature requires between 240ºC and 300ºC and pressures of 2-3 105 kPa for longer than 1000 years (Frimmel *et al.* 1993). However, Southam & Beveridge (1994, 1996) have shown that *Bacillus subtilis,* a common soil bacterium, was able to immobilise more then 100 µg g^{-1} (dry weight bacteria) as fine-grained intracellular colloids (5-50nm). The bacteria were killed in this process, autolysis was initialised, proteins were released and pseudocrystalline gold was formed, which at first formed roughly shaped noncrystalline octahedral gold, and was then transformed into crystalline octahedral gold (20µm) at low temperatures between 60ºC to90ºC within 4 weeks (Southam & Beveridge 1994, 1996)

Beveridge & Murray (1976) discovered that the reduction and precipitation of Au^{3+} from $AuCl_4^-$-solution was selective as other metals, such as Ag^+ were not reduced. In solutions containing Au^{3+}, Cu^{2+}, Fe^{2+} and Zn^{2+}, Au^{3+}, was selectively adsorbed by *Bacillus subtilis* and the cyanobacterium *Spirulina platensis* (Gee and Dudeney, 1988). The accumulation of ionic and colloidal gold was further investigated by Ulberg *et al.* (1986) and Karamushka *et al.* (1987a, b). They found that the accumulation of gold was dependent on the chemical structure of the cell envelopes, and involved functional groups of proteins and carbohydrates. In further studies they uncovered that the accumulation of gold was directly dependent on the metabolic activity

of the cell culture (Karamushka *et al.* 1990b, Ulberg *et al.* 1992). On a cellular level the accumulation of gold appears to be dependent on metabolic reactions on the plasma membrane, in particular the hydrolysis of ATP (adenosine triphosphate) by the enzyme ATPase. Various inhibitors were used to inhibit the ATPase activity and results showed that gold accumulation was dependent on the proper functioning of this enzyme (Karamushka *et al.* 1990b,c).

Energy dependent uptake of gold has also been shown for other microorganisms such as *Bacillus cereus* and *Spirulina platensis* (Savvaidis *et al.* 1997). Other organisms such as fungi, algae and yeast appear to precipitate gold by electrostatic interaction (Savvaidis *et al.* 1997). Addition of Au^{3+}-solution to cell suspensions of *Geobacter metallireducens* oxidized c-type cytochromes, which are thought to be involved in electron transport to metals (Lovely & Phillips 1988). Studies conducted with mesothermophilic and hyperthermophilic dissimilatory Fe (III)-reducing bacteria and archaea demonstrated that some of these organisms, such as *P. islandicum*, *S algae*, *D. vulgaris* and *G. ferrireducens*, are capable of precipitating gold by reducing Au^{3+} to Au(0) with hydrogen as electron donor (Kashefi *et al.* 2001). In these experiments gold was reduced and precipitated along the cell walls. Thus, these organisms living in hot environments of 60ºC to 110ºC might also contribute to the formation of gold deposits. In the Champagne pool, a large hot spring at Waitapu on New Zealands north island, thermophilic and hyperthermophilic bacteria and archaea have been shown to contribute to the formation of gold bearing sinters (Jones *et al.* 2001). Recently the bacterium *Hyphomnas adhaerens*, a species closely related to *Pedomicrobium*, which was shown to be involved in the formation of placer deposits, was shown to function as hyperaccumulator for gold (Quintero *et al.* 2001). *Hyphomnas adhaerens* was able to bind colloidal gold to the polar polysaccharide capsule of the prosthecate cell, whereas a mutant without the capsule was not able to do so (Quintero *et al.* 2001).

GOLD FLAKES FORMED BY MICROORGANISMS

The origin of gold flakes and nuggets has long been the subject of discussion among geologist studying placer deposits. Two theories currently prevail: one holds that the nuggets are formed mainly by chemical accretion process; the other maintains that they are of detrital origin (Boyle 1979). Evidence complied by Boyle (1979) suggest that nuggets are partly of chemical partly of detrital origin, because none of the theories mentioned above was capable to explain all phenomena occurring in placer deposits. As illustrated above compelling evidence exists for the influence of microorganisms on the geochemistry of gold, so it was not surprising when Watterson (1992) first reported gold encrusted microfossils on placer gold specimens from Lillian Creek in Alaska, USA. On these particles lacelike networks of micrometer-size filiform gold associated with the placer gold particles were interpreted as low-temperature pseudomorphs of a *Pedomicrobium*-like budding bacterium (Watterson 1992). The cells occurring in Lillian Creek gold were oval, spheroidal, and kidney shaped and the morphology that of *Pedomicrobium manganicum* closely. The pseudomorphs ranged between 0.9 and 1.5 µm in size, which is also the size range reported for *Pedomicrobium manganicum*. Colonies on the grains consisted of branching and anastomosing axial and lateral direct budding cells. *Pedomicrobii* reproduce by budding and thus are identifiable based on morphology (Staley 1971, Hirsch 1974, Gebers 1981). The budding growth enables *Pedomicrobium* to remain ahead of being encrusted into the gold it itself has accumulated (Mann 1992). Watterson (1992) studied 18,000 grains from different sites in Alaska and came to the conclusion that the majority of these grains included gold, which had been accumulated by bacteria.

In studies undertaken with samples form China, Venezuela and Australia similar results were obtained (Smith *et al.* 1991, Bischoff *et al.* 1992, Keeling 1993, Bischoff 1997). Keeling (1993) found gold encrusted cells on two thirds of the examined nuggets from Watts Gully in South Australia. The morphologies of these cells and colonies of cells were very similar to those described previously by Watterson. Numerous specimens from five locations in Australia have been studied by Bischoff (1995, 1997). He found that 70 to 100% of the crevices in placer gold particles were colonised by bacteria. Alluvial placer gold particles showed signs of transport and were colonised following post-lodgement chemical rounding, by budding and branching, gold adsorbing bacteria. They were represented by deposits of pure gold on their outer surface and were very similar to, though not identical with gold pseudomorphs of *Pedomicrobium*-like budding bacteria described by Watterson. In his article Bischoff (1997) describes an organically persevered filamentous organism, associated with the *Pedomicrobium*-like structures. Morphologies of its hyphae, sclerotium formation on the mycelium, and a positive test for the presence of polysaccharides in the hyphae suggested a fungal affinity. A DAPI-stain was conducted and the presence of DNA in the nuclei was confirmed, identifying the structure as part of a living organism (Bischoff, 1997).

MICROORGANISMS AS GEOMICROBIOLOGICAL INDICATORS FOR GOLD DEPOSITS

Previous studies have shown that some microorganisms may also indicate the presence of buried deposits

(Parduhn *et al.* 1985, 1991). The use of the soil bacterium Bacillus cereus as an indicator organism for gold and other metals has been explored in studies of different terrains in Belgium, China, Argentina and Mexico (Neybergh *et al.* 1991, Melchior *et al.* 1994,1996, Wang *et al.* 2002). Bacillus cereus is an aerobic spore-forming soil bacterium, which displays a high metal and penicillin tolerance. Its spores have been isolated from most soils and sediments in numbers of several hundred colony-forming-units (CFUs) per gram dry weight of soil (Watterson 1985). However, the spore counts in polymetallic-enriched soils, especially those with high gold concentrations, were up to several orders of magnitude higher than background, indicating an unambiguous association of bacterial population with polymetallic soils (Watterson 1985, Neybergh *et al.* 1991, Melchior *et al.* 1994, 1996, Wang *et al.* 1999). Due to their high oxidation potentials most Au(I)- and Au(III)-complexes display strong bactericidal properties even at low concentrations. (Karthikeyan & Beveridge 2002). Hence, mobile Au-complexes could be directly responsible for the increase of Bacillus cereus spores in gold-bearing polymetallic soils by suppression of competing species. Penicillin excreting fungi have been shown to be abundant in aurophilic and pollymetallic soils (Melchior *et al.* 1994). While most bacteria are killed by the penicillin released from fungi in the soil, Bacillus cereus can break down these antibiotics and convert them into other chemicals called chelates. Chelates then attach themselves to the particles of gold and other metals in the soil, preventing those particles from killing Bacillus cereus perhaps indicating why Bacillus cereus is noticeably enriched in these soils (Parduhn *et al.* 1985). However, if gold concentrations reach a level above the capacity of the gold chelating process to detoxify the ionic gold sporulation might be induced. In laboratory studies Bacillus cereus has been shown to react to elevated ionic gold concentrations by sporulating (Wang *et al.* 2002).

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