Cupriavidus metallidurans and the biomineralization of gold

The role of bacteria in the formation of secondary gold on grains in the Australian regolith

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Statement of Originality

'I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material written by another person except where due reference is made in the text.

Chapters 3 and 4 are published as Fairbrother *et al.*, 2013 and 2012 respectively. The co-authors provided advice and helped to write the manuscripts.

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Published journal articles from this thesis

Fairbrother, L., Brugger, J., Shapter, J., Laird, J.S., Southam, G., Reith, F., 2012. Supergene Gold Transformation: Biogenic Secondary and Nano-particulate Gold from Arid Australia. Chemical Geology, 320,17-31.

Fairbrother, L., Etschmann, B., Brugger, J., Shapter, J., Southam, G., Reith, F., 2013. Biomineralization of Gold in Biofilms of *Cupriavidus metallidurans*. Environmental Science & Technology, 47(6), 2628-2635.

Abstract

Cupriavidus metallidurans dominates bacterial communities in sheet-like biofilms on Australian gold grains. This organism has the capability to take up aqueous gold-complexes (Au-complexes), precipitating gold nanoparticles and is likely a main driver of gold biomineralization in the Australian regolith. Existing biogeochemical models for the formation of placer grains have placed importance on the role bacterial biofilms play in their genesis, and in the dispersion of gold throughout the environment. However, the importance of *C. metallidurans* remains to be adequately shown. This study demonstrates the ability of *C. metallidurans* to play a key role in the geomicrobiological cycle of gold by precipitating Au-complexes in planktonic and biofilm modes of growth in regolith-simulating experiments. Active versus inactive states of cell metabolism were compared in order to define the active uptake (*i.e.*, the definable removal of gold from solution by bacteria) and passive sorption (*i.e.*, retention) of Au-complexes across a pH range. In batch experiments with Au(I)-complexes a biologically active pure culture of planktonic *C. metallidurans* cells, over 144 hours, took up a maximum of 68.9 % of the gold, present as Au(I)-thiosulfate in media that contained the minimal nutrients possible for colony growth. In contrast, 17.5 %, 7.9 % and 3.6 % was passively adsorbed by the inactive, sterile and abiotic controls respectively under identical conditions. The gold complexes, Au(I)-thiomalate, and Au(I)-cyanide were also investigated however Au(I)-thiosulfate showed the greatest variation of uptake across cell states. Thus,

columns experiments designed to optimally support microbial growth (via the use of “full” media; a largely undefined, non-selective medium comprising of all the elements most bacteria need for growth) on sand grains, saw metabolically active biofilms of *C. metallidurans* take up ~100 wt.% of gold from increasing concentrations of aqueous Au(I)-thiosulfate solutions over 84 days. This uptake of gold from Au(I)-thiosulfate by active biofilms of *C. metallidurans* resulted in the formation of gold particles associated with cells and the associated exopolymeric layer. The formation of gold occurred as isolated nano-particles, conglomerates of nano-particles directly associated with cells, and as larger (> 1 µm) extracellular spheroidal and framboidal particles. Larger particles were rod-shaped and hollow confirming assumptions that cellular processes lead to the encapsulation and replacement of cells whilst preserving their morphology. Biofilm growth of *C. metallidurans* resulted in minimum bactericidal concentrations (MBC) six times that of planktonic *C. metallidurans* cells in aqueous Au(I)-thiosulfate. This capacity to take up and precipitate Au-complexes makes *C. metallidurans* a good fit for modeling supergene gold grain genesis that also encompasses the dispersion of gold. To determine the applicability of such a model and confirm the influences of (bio)geochemical processes on the transformation of gold grains in arid Australian environments, supergene grains from eight arid sites in three Australian gold provinces, *i.e.*, Lawlers (Western Australia), Tanami (Northern Territory), and Flinders Ranges (South Australia) were collected and characterized. Collection was based on

contrasting deposit styles, *i.e.*, primary underground and epithermal deposits as well as secondary elluvial-, colluvial- and alluvial placers at increasing distances from primary mineralization. Gold grains from all surface environments displayed supergene transformation features, including spheroidal and bacteriomorphic gold, which developed and increased with distance to source. While viable biofilms and gold nano-particles and spheroidal gold µ-crystals were detected on all grains from the Flinders Ranges, gold grains from the Lawlers and the Tanami provinces were found to be covered by a polymorphic layer in which nano-particulate, spheroidal and bacteriomorphic gold was dispersed. These polymorphic layers consist of materials suggestive of remnant biofilms and due to the similarities seen on grains from the field and within biofilms in the laboratory it is concluded that biofilms capable of transforming gold grains develop periodically on gold grains in arid environments. This study shows that microbial processes, particularly those of biofilms and *C. metallidurans*, play a critical role in the transformation of supergene gold grains and contribute to the dispersion of gold and geochemical anomalies in arid environments.

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