

***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

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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.

Table of Contents

Declaration	ii
Published journal articles from this Thesis	ii
Acknowledgements	iii
Abstract	vii
Table of Contents	x
List of Figures	xiii
List of Tables	xvii
List of Abbreviations	xviii
Chapter 1: Introduction	1
1.1. Gold and its presence in the supergene	1
1.2. Microbially mediated solubilization and mobilization of gold in supergene environments	4
1.3. Microbial precipitation of gold complexes	8
1.4. <i>Cupriavidus metallidurans</i> ; gold resistance and inherent advantages	16
1.4.1. <i>Cupriavidus metallidurans</i> biofilms on gold grains	17
1.4.2. <i>Cupriavidus metallidurans</i> and the formation of secondary gold structures	19
1.5. Formation theories of secondary structures on primary grains	21
1.6. Aims, hypotheses and objectives	25
Chapter 2: Uptake of gold from Au-complexes by <i>Cupriavidus metallidurans</i>	29
2.1. Introduction	30
2.2. Materials and methods	34
2.2.1. Bacterial growth	34
2.2.2. Gold retention experiments	37
2.2.3. Analysis of solutions	39
2.3. Results and discussion	40
2.3.1. Gold uptake from Au(I)-thiosulfate by <i>Cupriavidus metallidurans</i>	40
2.3.1.1. Retention of gold from 500 µM Au(I)-thiosulfate in full medium	41

2.3.1.2.	Uptake of gold from 500 μ M Au(I)-thiosulfate in minimal medium	43
2.3.2.	Gold uptake from Au(I)-thiomalate by <i>Cupriavidus metallidurans</i>	49
2.3.2.1.	Retention of gold from 500 μ M Au(I)-thiomalate in full medium	49
2.3.2.2.	Retention of gold from 500 μ M Au(I)-thiomalate in minimal medium	51
2.3.2.3.	Uptake of gold from 50 μ M Au(I)-thiomalate in minimal medium	53
2.3.3.	Retention of gold from Au(I)-cyanide by <i>Cupriavidus metallidurans</i>	62
2.3.3.1.	Retention of gold from 500 μ M Au(I)-cyanide in full medium	62
2.3.3.2.	Retention of gold from 500 μ M Au(I)-cyanide in minimal medium	63
2.3.4.	Gold uptake from 0.5 μ M Au(I)-complexes by <i>Cupriavidus metallidurans</i> in the absence of nutrients	66
2.4.	<i>Cupriavidus metallidurans</i> ; active and passive adsorption and the biomineralization of supergene gold	68

Chapter 3: Biomineralization of gold in biofilms of *Cupriavidus metallidurans* **70**

3.1.	Introduction	71
3.2.	Materials and methods	74
3.2.1.	Column experiments	74
3.2.2.	Analyses of outlet solutions	77
3.2.3.	Analyses of solid phases	78
3.3.	Results and discussion	80
3.3.1.	Uptake of Au(I)-thiosulfate in columns	80
3.3.2.	Assessment of bacteria and associated gold biominerals in outlet solutions	84
3.3.3.	Distribution of gold in biofilms	86
3.3.4.	The role of biofilms in gold biomineralization	92

Chapter 4: Supergene gold transformation: biogenic secondary and nano-particulate gold from arid Australia **96**

4.1.	Introduction	97
4.2.	Field sites	104
4.2.1.	Lawlers Tenement, Western Australia	104
4.2.2.	Old Pirate prospect, Tanami Gold Province, Northern Territory	107
4.2.3.	Lively's Find, Northern Flinders Ranges, South Australia	109
4.3.	Materials and methods	110

4.3.1. Sample collection and preparation	110
4.3.2. Analysis of gold grains	110
4.4. Results and discussion	112
4.4.1. Primary gold from underground and surface exposed quartz-vein systems	113
4.4.2. Gold grains from placer environments	119
4.4.2.1. Proximal grains	119
4.4.2.2. Distal grains	130
4.4.3. Biofilms: key-catalysts for the supergene transformation of gold grains	135
4.4.4. Application to mineral exploration	139
Chapter 5: Conclusions – <i>Cupriavidus metallidurans</i> plays a key role in the formation of secondary gold grains found in the Australian regolith	141
5.1. Uptake of environmentally relevant Au(I)-complexes, by <i>Cupriavidus metallidurans</i> and the effects of cell state	142
5.2. <i>Cupriavidus metallidurans</i> mediated precipitation of Au(I)-complexes in supergene settings	144
5.3. Biomineralization and the accumulative effect of microbial surface processes on placer grains in Australia	146
5.4. An updated model for the microbially mediated formation of gold grains in the Australian regolith	148
5.5. Applications and limitations of the proposed model and future research directions	151
5.6. Future applications of this research	164
References	166