# EXPLORATION GEOMICROBIOLOGY – THE NEW FRONTIER

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## INTRODUCTION

Microbial processes are known to influence the mobilization, distribution and speciation of many trace metals (*e.g.*, As, Se, Mo, Sn, Sb, Te, Hg, W, Cd, Hg, Pb, U, Ag, Cu and Au) under a wide range of environmental conditions common in the regolith (Ehrlich 1996a,b; Reith & McPhail, 2006; Reith *et al.*, 2006). Iron- and sulfur-oxidizing bacteria and archaea mediate the oxidative breakdown of sulfide minerals and thus, contribute directly to the dispersion of metals associated with these minerals (Nordstrom & Southam, 1997). Organic, inorganic acids and cyanides excreted by microorganisms form stable complexes with many trace metals (Korobushkina *et al.*, 1983; Faramarzi & Brandl, 2006). Bacteria and fungi also promote the formation of silicate, carbonate and sulfide minerals, as well as mediate metallic mineral formation by binding metal cations to negatively charged groups of the cell all or cell envelope (Ehrlich, 1998). In turn, the structure and activity of microbial communities resident in the regolith are strongly influenced by the prevailing geochemical conditions, such as pH, redox conditions, organic matter and trace element contents (Ehrlich 1996a,b; Kizilkaya *et al.*, 2004). To generate an understanding of the relationship between the microorganisms in the regolith, their geochemical environment and processes relevant to mineral exploration, exploration geomicrobiology has emerged as a new area of research.

## KEY OBJECTIVES OF EXPLORATION GEOMICROBIOLOGY

Exploration geomicrobiology provides an understanding of microbial populations and processes required to successfully develop bio-prospecting tools and predictive biogeochemical models. Figure 1 illustrates the integrated approach used to identify geomicrobial processes and associated microbial populations mediating metal dispersion and re-concentration in the regolith. This knowledge can then be applied in the development of bio-prospecting tools such as molecular or microbial biosensors, as well as predictive modelling applications. To understand these key processes and populations in the Australian regolith, exploration geomicrobiology has to address the following key questions:

- Are geomicrobiological processes leading to trace metal dispersion and re-concentration ubiquitous in the Australian regolith?
- Which zones of the regolith harbour microorganisms that directly influence trace metal cycling?
- Which key processes and organisms are responsible for geomicrobial transformation of trace metals?
- What are the genetic, physiological, and biochemical mechanisms involved?
- How do environmental conditions influence the activity of these microorganisms?
- What are the kinetics of trace metal solubilisation, transport and precipitation of trace metals under *in situ* conditions in the regolith?
- In which form are the trace metals elements transported, as free ions, organic or inorganic complexes or colloids?
- Which microorganisms are closely associated with mineralisation and can be used as biomarkers or biosensors?

Developing a mechanistic understanding of trace element dispersion and re-concentration in regolith materials, which display complex inorganic and organic matrices and distinct abiotic and biotic phases, is challenging. Understanding the biotic phases in these systems is particularly demanding, because one gram of regolith material may contain several thousand different microbial species of which 99% have not yet been identified (Paul & Clark, 1996). Furthermore, regolith materials harbour large numbers of microsites, which have different biogeochemical characteristics (*e.g.*, pH, redox potential, water content, organic substrates, metal concentrations or xenobiotics) and as a result differing microbial communities compared to the surrounding bulk soil. These microsites are often characterised by enhanced biogeo-chemical cycling of elements (*e.g.*, Campbell *et al.*, 1989; Peters & Conrad, 1996; McClain *et al.*, 2003; Bruelheide & Udelhofen, 2005).

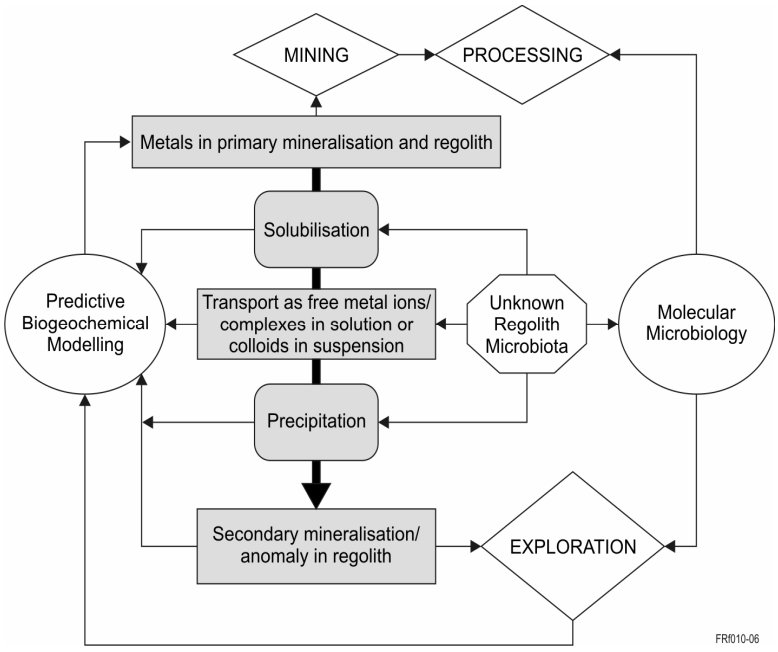


**Figure 1.** Schematic diagram illustrating links between geomicrobial processes (in grey) with areas of research and the requirements of the minerals industry.

**METHODOLOGY USED IN EXPLORATION GEOMICROBIOLOGY**

To study the diversity of complex microbial populations as well as their functional activity in the regolith, numerous methods have been developed, which are grouped into culture-dependent and culture-independent techniques (Figure 2; Barns & Nierzwicki-Bauer, 1997). Culture-dependent techniques use growth media to enrich and isolate microorganisms from regolith samples (Barns & Nierzwicki-Bauer, 1997). However, all culture-dependent methods share one main bias: they rely on having to culture the organisms in a growth medium *in-vitro*. Depending on the type of regolith sample only 0.001 to 10 % of the total microbial species contained therein can be successfully cultured using existing culturing techniques (Alexander, 1977).

In recent years culture-independent methods of characterizing microbial population in regolith samples have been developed. These methods are based on the analyses of cellular components such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA) or phospholipid fatty acid (PLFA) (Olsen *et al.*, 1986; Pace *et al.*, 1986; Barns & Nierzwicki-Bauer, 1997). These cellular components are extracted directly from regolith materials without prior cultivation. The main advantage of molecular methods is that they do not rely culturing the organisms and thus, provide a more accurate insight into the *in situ* composition and activity of microbial communities in regolith samples. Figure 2 shows how they are used in sequence/combination to obtain a detailed representation of the phylogenetic and functional relationships in microbial communities. Phylogenetic methods aim to identify key-organisms, community structures and genetic relationships and are based on the extraction, amplification, fingerprinting, sequencing and analyses of, typically, ribosomal DNA and RNA (Barns & Nierzwicki-Bauer, 1997). Methods assessing the functional aspects of microbial communities target genes that encode proteins/enzymes responsible for key biogeochemical transformations.

Identification of the microbiota and microbial processes that control the solubilization, transport and precipitation of trace metals in the regolith may lead to the discovery of microbial indicator organisms and genes, and the development of microbial biosensors for underlying mineralization (Reith *et al.*, 2005). Using signatures of genes involved in metal cycling will lead to the development of specific nucleic acid probes and microarrays for these bio-indicators that can be used directly for bio-prospecting. Molecular microarrays are gene-chips on which several hundred thousand nucleic acid probes can be placed and used simultaneously. Heavy metal specific bacterial sensors are another promising bio-prospecting tool, which allow to measure the concentration of mobile heavy metals in soil samples to µg $kg^{-1}$ (ppb) levels (Tibazarwa *et al.*, 2001; Rensing & Maier, 2003). However, for this to become reality molecular microbial biosensor and bio-indicator methods must first demonstrate their full potential as well as their cost competitiveness and benefit over existing methods.

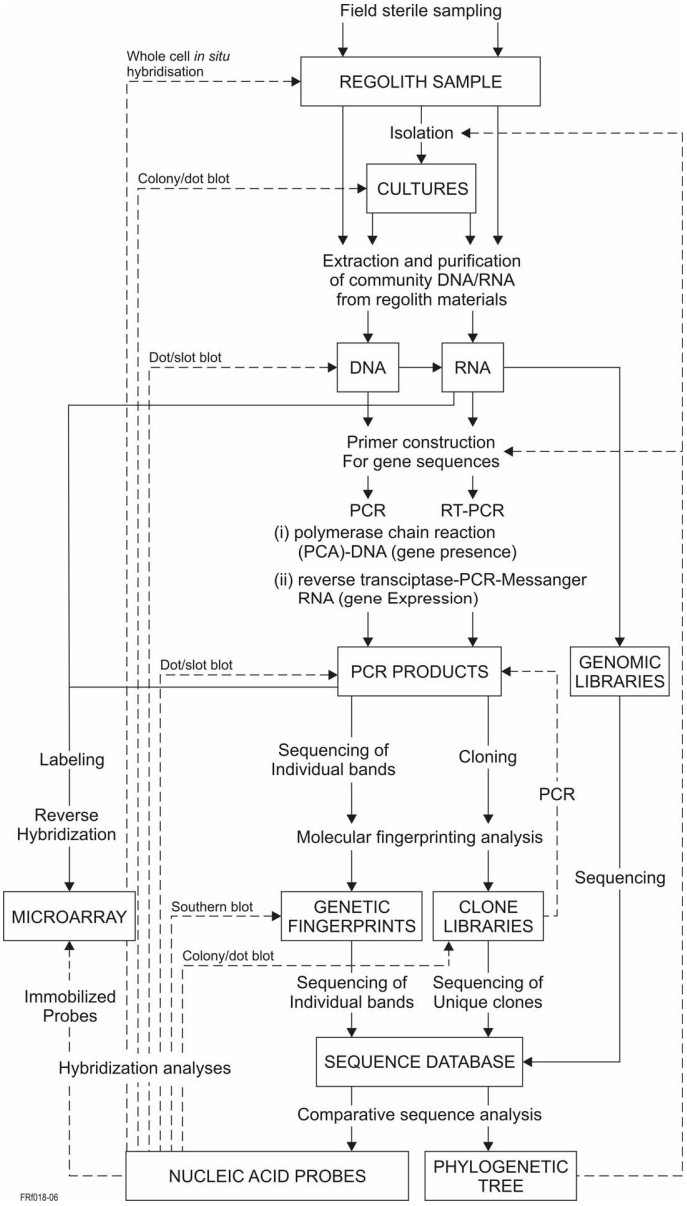


**Figure 2**
Molecular approaches for detection and identification of microrganisms involved in cycling of heavy metals and their catabolic genes from environmental samples (adapted from Widada *et al.*, 2002).

**BIOMEDIATION OF GOLD IN CALCRETE ANOMILIES – A CASE FOR EXPLORATION GEOMICROBIOLOGY**

An example demonstrating how exploration geomicrobiology is already used to understand the formation of regolith materials important for geochemical exploration is research conducted into the microbially mediated formation of gold anomalous carbonates (Schmidt Mumm & Reith, in press). The genesis of pedogenic carbonates has been ascribed to abiotic and biologically mediated precipitation in regolith profiles, and both mechanisms have been observed in the natural environments (Castanier et al., 1999a,b; Stumm & Morgan, 1996). Mass balance estimates by Castanier *et al.* (1999a,b) suggest that the heterotrophic microbially mediated carbonatogenesis accounts for the most of the formation of these carbonates rather than abiotic processes. One of the most common microbial processes leading to the formation of carbonate is the degradation of urea (Castanier *et al.*, 1999). Urea is a product of the metabolic breakdown of purine and amino acids in bacterial cellular processes through which microorganisms derive nutrition and energy (Vogels & Drift, 1976; Cunin *et al*., 1986).

Recently, Schmidt Mumm & Reith (*in press*) investigated the formation of gold anomalies in calcrete (2.5 to 50 ppb) and microbial degradation of urea in regolith carbonate accumulations in aeolian sand dunes of 2 to 4 m thickness overlying gold mineralisation at the Barnes gold-in-calcrete anomaly in South Australia. The results of their microbial study suggested that the genesis of calcrete may be biomediated through the

microbially mediated urea decomposition, which occurred in all samples in a depth profile and potentially provided a pH and $pCO_2$ environment conducive to carbonate precipitation. Schmidt Mumm & Reith (*in press*) proposed a coupled model of bio-mediated and inorganic mechanisms that control gold and calcrete precipitation as shown in Figure 3. If the amino acid breakdown can be applied to those involved in gold complexing, the process would have a destabilising effect on the gold-amino acid complexes (Reith & McPhail, 2006). The urea released during the break down of these amino acids is transformed by the urease to $CO_2$ and $NH_3$ establishing pH and $pCO_2$ (or $aCO_3^{2-}$) conditions conducive to biologically mediated carbonate precipitation in the calcrete bearing environments. Together with the destabilised gold amino acid complexes this process potentially provides a tight link between carbonate formation and related gold enrichment in calcretes.

Recent laboratory experiments have corroborated this model. Microbial enrichment cultures obtained from the calcareous materials from three depths (10, 64 and 210 cm) overlying the mineralization at Barnes were incubated in urea and $CaCl_2$ containing microbial growth media (pH 8) amended with 100 ppb of a gold as an aspartic acid complex. Within 96 to 240 h from the start of the incubation the urea was turned over to $NH_4^+$, the pH in solution rose by approximately 1 unit to pH 9 and $Ca^{2+}_{aq}$ was precipitated small Ca-carbonate crystals. Gold was uniformly dispersed in these carbonates crystals as shown by laser ablation ICP-MS and enriched by a factor of up to 800 times compared to the surrounding microbial growth medium. These results demonstrate that microrganisms resident in the calcareous materials at Barnes are capable of forming Au anomalous Ca-carbonates, which supports the model shown in Figure 3. In further steps DNA was extracted from natural samples as well as enrichment cultures, fingerprinting analyses can be conducted to identify the organisms involved. Furthermore, we will establish the kinetics of the processes in the in vitro and in situ experiments to link the microbial carbonate precipitation with controlling environmental factor as well as the ages of carbonates.

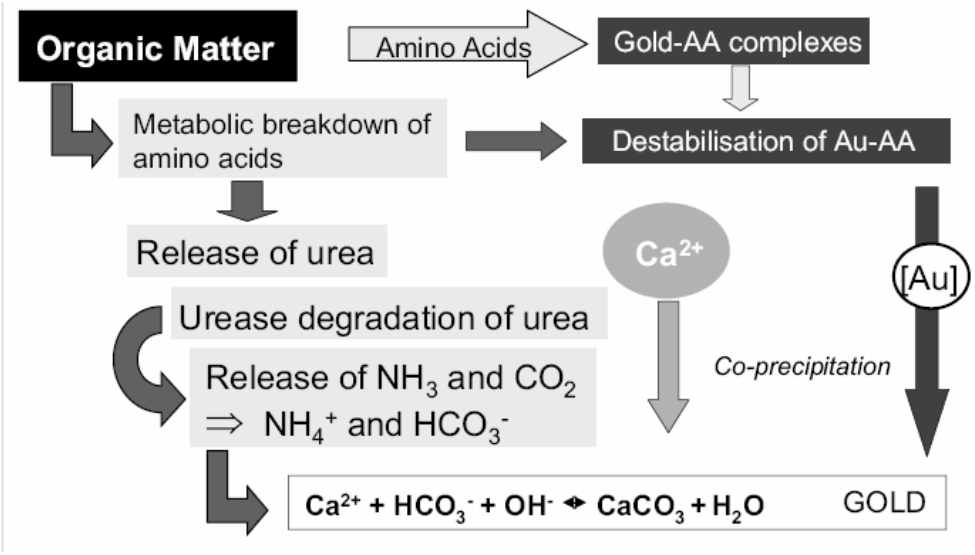


**Figure 3** A coupled biologically mediated and abiotic model for the Au enrichment in calcrete (from Schmidt Mumm & Reith, in press).

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