DETERMINING THE ROLE OF BIOLOGICAL MECHANISMS IN MINERAL TRANSFORMATIONS AND TRANSPORT IN THE AUSTRALIAN REGOLITH-PUTTING THE 'BIO' BACK INTO 'BIOGEOCHEMISTRY'

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Recent work in the CRC has for the first time categorically demonstrated the key role of bacteria in the solubilisation and precipitation of Au in the Australian regolith, the role of microbial and plant organic acids in mineral weathering around the roots of native eucalypt species, and is further elucidating the role that bacteria play in the oxidation and reduction of sulfidic materials. Biogeochemical research in CRC LEME is developing a sound mechanistic understanding of the principle biological processes underpinning biogeochemical dispersion, mobilization, transport, and mineralisation of minerals & trace elements in the Australian regolith, as well as determining the relative role of biological and non-biological (abiotic) processes driving mineral transport. The core aim of LEME activities in this area is the development of predictive models to identify where in the regolith biological mineral transformation and transport processes occur.
Microorganisms are likely to have played a significant role in the formation of the Australian regolith and in the transport and transformation of minerals within regolith materials. Single celled bacteria were the only forms of life on the planet for approximately 50% of earths history, first appearing about 4000 x 10^6 years before present (BP). More complex organisms such as fungi first appeared around 2000 x 10^6 years BP. Fossil records suggest that these early single celled organisms had similar cell structure to modern bacteria. In contemporary regolith environments about 5000 microbial species have been isolated and identified, with one gram of surface regolith material containing between a million and a billion cells. However, estimates of the total number of species in the regolith range from 100,000 to 1 x10^6, suggesting up to 95% of regolith microorganisms are unknown to science.

Geochemical transformations resulting from microbial activity in the regolith are also well established. One of the most well known is the biological oxidation of sulfide minerals, carried out by a group of bacteria known as chemolithoautotrophes. These organisms are highly adapted to low pH (highly acid) environments, and utilise Fe or S as part of their energy generating processes, catalysing Fe and S oxidation through the production of iron or sulfur oxidase enzymes, bacteria are therefore the key biochemical catalysts of geochemical transformations in these environments. Bacteria responsible for the oxidation of sulfide minerals including As, Cu, Co, Fe, Ni, Mo, Pb, Zn have been isolated from sulfidic environments and historically classified as members of the Thiobacillus genus notably (*Thiobacillus* sp., *Acidothibacillus* sp. *Sulfobacillus thermosulfidooxidans* and *Acidanus brierleyi*). (Mukhopadhyaya *et al*., 2000).

BIOLOGICAL ACTIVITY AND BIOGEOCHEMICAL TRANSFORMATIONS

Much of the current approach to the study of microbial transformations of minerals has focussed on the identification of organisms, species diversity and population dynamics, (Baker and Banfield, 2003) and their relationship to rates of mineral transformation. In fact environmental microbiology research concerning defining the role of biota in driving biogeochemical transformations in general has focussed on establishing the relationship between bacterial diversity and rates of biogeochemical processes (Rogers and Colloff, 1999). Unfortunately, traditional microbiological techniques applied to the study of regolith microbial populations fail to identify more than 5% of the species present. They also fail to determine the 'functional attributes' of microbial populations responsible for geochemical transformations.

APPLICATION OF NEW MOLECULAR BIOLOGY TOOLS

CRC LEME is developing an alternative approach to determining the mechanistic relationship between microbial activity and biogeochemical transformations, targeting the 'molecular genetic' attributes of microbial populations (Rogers *et al.* 2002). We have developed a new generation of molecular biology techniques to study the role of biota in mineral biogeochemical transformations, based on the

direct extraction of nucleic acids (DNA and RNA) from regolith samples that remove the need to isolate and culture individual organisms. They allow direct comparison of biogeochemical reaction kinetics, gene expression and biotic diversity of organisms, all in the same sample, and represent a major advance over traditional microbiology methods. A major advantage of these techniques is the rapid analysis of samples, to determine microbial attributes responsible for mineral transformation, and identify organisms responsible. Molecular techniques can provide details within a matter of days, compared to weeks or months when traditional microbiology techniques are used.

Examples of the application of molecular tools to the study of Regolith biogeochemical mineral transformations

i. Identification of chemolithotropic microbial functional genes involved in Sulfide mineral oxidation

The suite of genes encoding for sulphide mineral oxidation in bacteria has recently been identified. The *sox*B gene encodes for a diheme cytochrome c enzyme and has been shown to be essential for chemolithotrophic sulfide mineral oxidation. This functional gene has also been identified in representatives of all known groups of chemolithotrophic sulfur oxidising bacteria, making it an ideal candidate for our functional molecular approach. Polymerase chain reaction (PCR) techniques have been designed that amplify the *sox*B gene sequence (Petri *et al*. 2001), allowing us to study of sulfur oxidising chemolithotrophic bacteria activity and presence in sulfidic sediments.

In brief, DNA and RNA were extracted from sulfidic sediments using established techniques. *sox*B gene sequences were amplified in sample DNA extracts. The presence of functional genes indicates the presence of the biological potential for S mineral oxidation reactions. Figure 1 summarises the detection of the *sox*B gene in sulfidic sediments actively oxidising in the River Murray Floodplain.

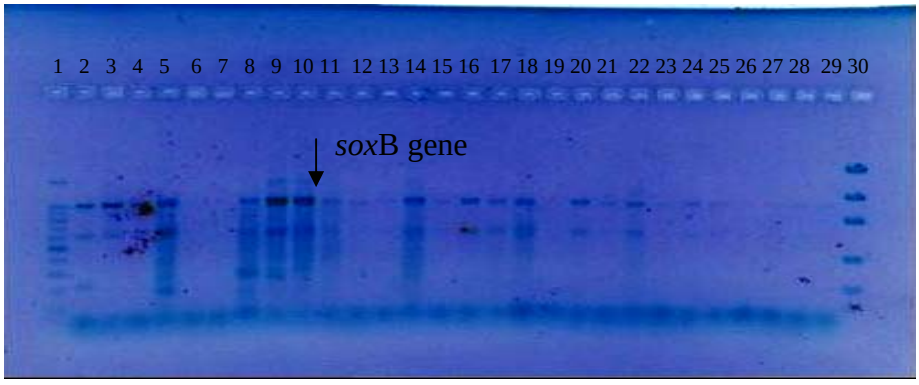

Figure 1 *soxB* functional gene DNA detected in extracts from sulfidic sediments . 1000 bp PCR product visualised on 2% agarose gel. **Lane 1** 100bp DNA ladder; **Lane 2-4** *A.caldus* soxB positive control 1000bp product; **Lanes 5-29** presence of *sox*B gene in sulfidic sediments; **Lane 30** DNA quantification marker.

ii. Microbial ecology/population diversity of Bacteria Associated with Gold solubilisation and precipitation

A current LEME PhD project has identified the role of bacteria in gold precipitation and solubilisation. In order to identify microorganisms present on gold flakes, numbers of species and if the organisms are living, the molecular technique 16S rRNA Density Gradient Gel Electrophoresis (DGGE) analysis has been used (Muyzer, 1999). In brief a region of the bacterial 16S gene can be amplified in DNA extracts. PCR products are separated on a DGGE electrophoresis gel, which separates double stranded DNA. Each different bacterial species has a unique 16S gene sequence, therefore each band on the gel represents an individual bacterial species. Individual sequences (bands) from the DGGE gel can be further analysed by sequence analysis in order to identify the species present. This technique allows the analysis of all organisms present, as no culturing and isolation of cells is required. Figure 2 shows a representative DGGE acrylamide gel showing bacterial species 16S gene diversity in DNA extracted from a single gold flake collected from the Tomakin Park gold mine.

The successful extraction and amplification of DNA from single gold flakes, confirms that the microbial biofilm thought to be responsible for solubilisation and precipitation reactions is 'alive'. DNA sequencing, the determination of the individual species DNA structure has identified the bacteria responsible for Au precipitation/solubilisation in auriferous regolith materials. This same species of bacteria is responsible for Au biomineralisation in both sites studied (Queensland and NSW).

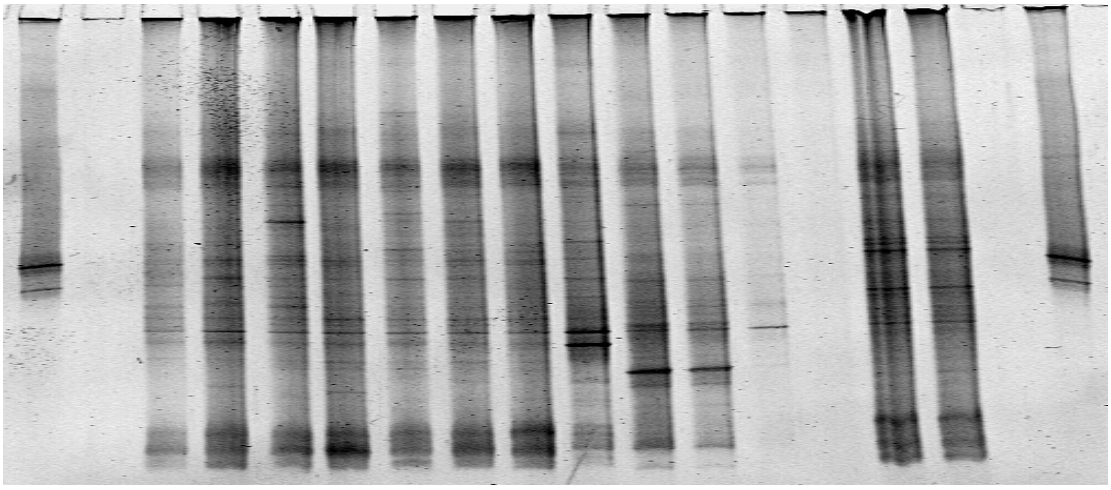

Figure 2. 16S rRNA Density Gradient Gel Electrophoresis profiles of bacterial populations on gold flakes collected at the Tomakin park gold mine. 16S V3 region amplified in soil DNA extracts with degenerate oligonucleotide PCR primers 27FGC and 534R. PCR product visualised on a 35-60% urea/formamide acrylamide gel (8%), 120V 20hrs. Each band on the gel represents an individual species of bacteria

Both these CRC LEME sponsored studies have demonstrated the application of new molecular techniques to the study of biological mineral transformations in the regolith.

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