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ESRF

Experiment Report EC-1069

ESRF	**Experiment title:** *Metalloenzymes as tracers of the evolution of early life: a study of transition metals and Br geochemical cycling in modern and ancient mats*	**Experiment number**: EC-1069
Beamline: ID22NI	**Date of experiment**: from: 21/11/2012 to: 25/11/2012	**Date of report**: 28/02/2014
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INTRODUCTION

Stromatolites are large-scale, potentially cyanobacterial induced, sedimentary structures that formed throughout Earth history since at least 3.5 Ga (billion years) ago. Microbial mats participate in the build-up of stromatolites through their metabolic activities that can result in the dissolution and precipitation of minerals. They also produce exopolymeric matrix (EPS) that are crucial structures for carbonate minerals nucleation and growth.

Microbial mats growing on modern stromatolites depend on metallic cations (i.e.V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo and W) and their ability to catalyse multi-electron redox and hydrolytic transformations essential for their life processes. In addition, secretion of exopolymeric matrix (EPS) by bacteria can trap and scavenge metals passively (Konhauser and Urrutia 2009). Therefore, trace metals can be employed as biomarkers for specific types of metabolisms in ancient kerogenous laminae, if secondary adsorption processes attending diagenesis and metamorphism can be eliminated. Iron has been extensively studied as a biomarker (Beard et al., 1999, Johnson et al., 2008). Other enzyme-based metals such as Cu, Co, Ni, Mo and As remain poorly studied and until recently, they were barely analyzed for their abundances (Anbar, 2008, Konhauser et al., 2011) and isotopic compositions (Zhu et al., 2002, Cameron et al., 2009).

An analytical protocol is required for unraveling the respective imprint of microbial vs diagenetic processes and to document the characteristic abundance, distribution and speciation of metals within the organic fraction present in modern and ancient mats. As part of experiment EC1069, two types of samples of different ages and biological origins were studied: (1) modern stromatolites from the Bahamas containing consortia of phototrophs, sulfate reducing bacteria, methanogens, which display different degrees of diagenesis; (2) 2.7 Ga old stromatolites from the Tumbiana Formation (W-Australia). This formation consists of a succession of carbonated stromatolites and mudstones containing two reservoirs of carbonaceous material with $\delta^{13}C$ values of -30‰ (oxygenic or anoxygenic photosynthesis) and -55‰ (methanotrophs).

Before accessing ID22NI, the samples were investigated for the distribution of transition metals using the ultrafast (µs dwell time/pixel) 384-element MAIA detector at the µ-XRF line at the Australian Synchrotron. This allowed collecting elemental maps of several cm² sample area with 2-3 µm resolution. Detailed investigations of these maps by Principal Component Analysis allowed identifying microscopic zones of interests where to concentrate the effort during experiment EC-1069.

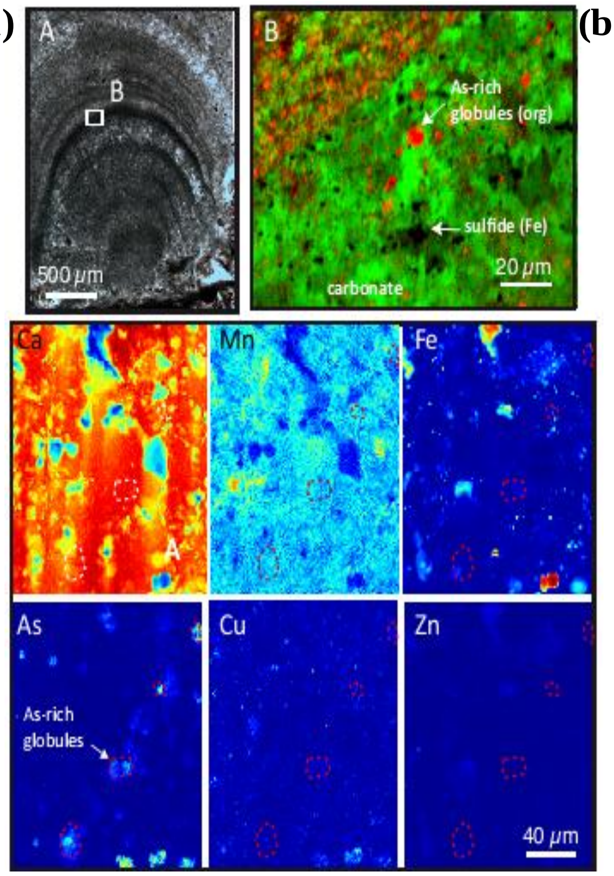
EXPERIMENTAL SETUP

ID22NI hard X-ray nanoprobe station was used at 15-16 kev excitation energy. Draft scanning (300 nm, 0.1 s/pixel) was carried out on previously identified zones of the Bahamian and Tumbiana stromatolites. This was followed by high resolution mapping (< 100 nm) at 1 s/pixel measurement time of specific areas identified from the draft maps. This analytical procedure has permitted to identify biogenic signatures and possible metabolic imprints in ancient microbial mats.

RESULTS

The high spatial resolution mapping of Tumbiana stromatolites have demonstrated the presence of Ca, Mn, Cu, Zn, Fe and As distribution at the µm-scale (**figure 1a**). The X-ray fluorescence map of various elements, shows intense abundancy of Ca and Mn associated with the mineral host forming the stromatolitic bulbs; Ca-carbonate. Cu and Zn show low abundances throughout the sample. However, Fe occurs concentrated in hotspots as randomly distributed grains, forming pyrite (FeS_2). Arsenic show the same type of hotspot distribution as Fe, but associatd either to Fe, forming arseno-pyrite (FeAsS), or to µm-scale organic globules, forming As-bearing organic globules, with no or only minor amounts of Fe and other trace metals (**Figure 1a**). Although pyrite and arseno-pyrite occur in all types of lithologies (mudstone layers, carbonate stromatolites and volcano-clastic sediments), As-bearing organic globules were only found in well-preserved micrite forming the stromatolite bulbs. Raman spectrometry analysis, showed that the As globules coincide with well preserved organic carbon (aromatic, aliphatic and carboxylic) attributed to microbial remains (Lepot et al., 2008). Accordingly, these cell-like and As-bearing carbonaceous globules identified in the 2.72 Ga old Tumbiana stromatolites may be considered as bio-indicators of an ancient As metabolism.

(a)



(b)

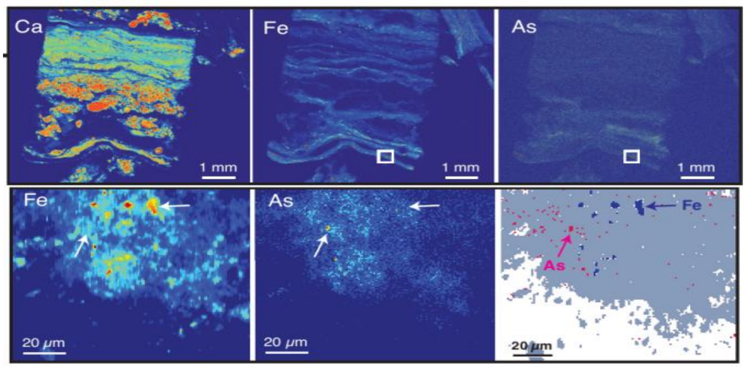


Figure 1. (a) Ca, Mn, Fe, As, Cu and Zn distribution maps in Tumbiana stromatolite. X-ray fluorescence distribution maps Ca, Mn, Fe, As, Cu and Zn in Tumbiana stromatolite. Cell-like As bearing globules occur in specific regions in the sample which is not correlated to Fe (forming pyrite) or to Cu and Zn **(b)** Ca, Fe and As distribution in modern stromatolites from the Bahamas (Highborne Cay). The figure demonstrates the enrichment of As in organic microglobules in both modern and fossil stromatolites. This elucidates to arsenic metabolism by microorganisms that could have thrived for over 2.7 Ga.

In modern stromatolites, the nm-scale imaging have demonstrated that most metals (Fe, As, Cu, Zn) are homogenously distributed throughout the organic layer forming the living mats with an exceptionally microscopic cell-like globules of pure As, occuring at specific areas of the microbial mats (**figure 1b**).

The presence of cell-like structures enriched in As and their association to organic matter in both modern and fossil stromatolites may reflect a pristine biosignature linked to the As cycle. Despite of its toxicity, arsenic can be readily metabolised by certain microbes in a various means of enzymatic interactions including; assimilation, methylation, detoxification and anaerobic respiration. Actually, this type of As/microbe interaction does exist in several microbial niches thriving in extreme environments such as hypersaline lakes lacking oxygen, where arsenic can offer a substitute for oxygen. In fact, there are strains of microorganisms isolated from the hypersaline Searles Lake, California that have shown to survive on arsenate as electron donor for respiration (Oremland et al., 2005).

The Tumbiana stromatolites had been been accreted in evaporative lakes formed on flood basalt. The saturated salt brines of the Tumbiana could had created an environment where sulfate reduction and methanogenesis were highly constrained. In the view of the fact that primitive Earth was devoid from molecular oxygen, abiotic oxidation of As could be excluded. So, arsenic is most reasonably present in its reduced form; arsenite As(III). For that reason, the most probable reducing substrate for microbial metabolism that could have been present in a such ancient lacustrine habitat is arsenite As(III). To that end, two types of arsenite oxidizing metabolism could had taken place on the Archean Earth; anoxygenic photosynthesis, or denitrification using As(III) as electron donors. In the presence of light, photosynthetic bacteria could drive anoxygenic photosynthesis metabolism producing energy through the oxidation of arsenite As(III) into arsenate As(V) (Engel et al., 2013). In the absence of light, another reasonable metabolism could had also occured, is the nitrate-linked anaerobic oxidation of arsenite As(III) achieved by chemoautotrophic bacterium using As(III) as electron donor (Rhine et al., 2007).

The major discovery unravelled in this study (experiment EC-1069), is the presence of As in microglobules of organic material in the well preserved micrite part of the 2.7 Ga old stromatolites. The findings of microbial remains composed almost exclusively of arsenic in the Tumbiana hypersaline lakes suggest that microorganisms living on arsenic could have evolved very early on an ancient anoxic Earth and most probably on similar hostile planets such as Mars and Europa (Sforna et al., in review). This accentuates the necessity to complete our study with the determination of different oxidation states of As in the discovered As bearing microglobules, to ascertain the presence of different types of ancient microbial metabolisms.

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