**Master Thesis in biology, microbiology**

Title (working title): **Enrichment and characterisation of bacterial diversity in Fe-rich hydrothermal deposits at Vail Lili and Mariner vent sites in Tonga Back Arch basin**

Tutors:
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Research group:
Geomicrobiology

Iron is one of the most abundant elements in the Earth's crusts. Iron has two main red-ox states in nature, reduced ferrous ($Fe^{2+}$) and oxidized, ferric ($Fe^{3+}$) form (Figure 1). A large part of this iron is in the ferrous form, which is in disequilibrium with the oxic atmosphere and thus represents a potential energy source for microorganisms.

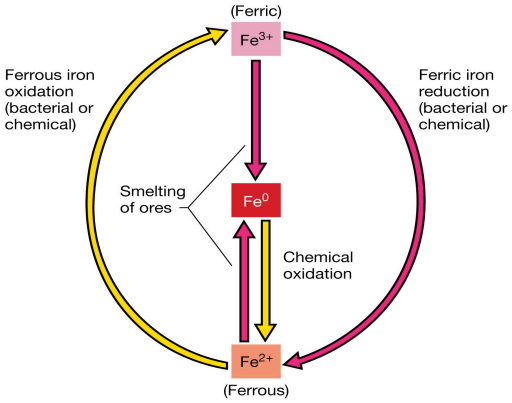
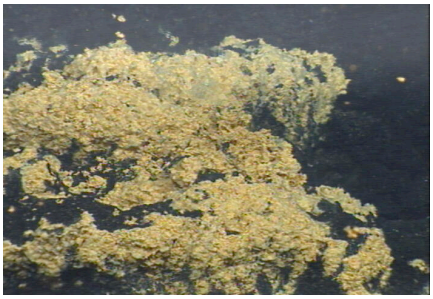



**Fig. 1 The redox cycle of iron. This figure shows that the major forms of iron in nature are $Fe^{2+}$ and $Fe^{3+}$. $Fe^0$ is generated as a product of human activities as melting of iron ores. Ferrous iron oxidation occurs aerobically by iron chemolitoautotrophs (figure adapted from Brock Biology of microorganisms by Madigan and Martinko 2006)**

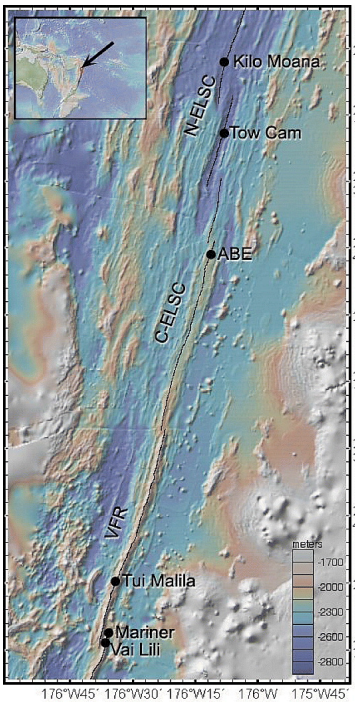
Iron plays an important role in living cells, often catalyzing electron transfer reactions. Some organisms also use iron in their energy metabolisms, either as electron acceptors (the iron reducing microorganisms) or as electron donors (the iron oxidizing microorganisms). The ferrous iron oxidizing bacteria (FeOB) can be divided into two groups, based on their use of electron acceptors, the aerobic and the anaerobic iron oxidizers. The first group use $O_2$ as the terminal electron acceptor while the latter use another acceptor e.g. $NO_3^-$. The aerobic iron oxidizers can be further divided into acidophilic and neutrophilic FeOB. This division is rational since $Fe^2+$ is only stable at pH < 4 in atmospheric equilibrated aqueous phases. At higher pH values iron quickly oxidizes to iron oxides and hydroxides. The rapid auto oxidation of reduced iron makes the study of neutrophilic FeOB in oxic environments particularly challenging. The neutrophilic aerobic iron oxidizing microorganisms are difficult and demanding to culture and consequently we know little about these organisms and their function in the global iron cycle.

Seafloor iron oxide deposits are a common feature of submarine hydrothermal systems and often large areas of vent fields are dominated by low temperature iron mound formations (see figure below). Only recently have studies begun to

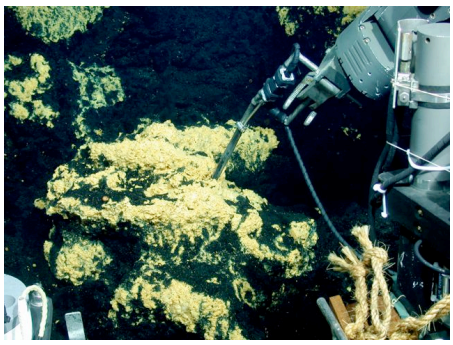
elucidate these processes and describe the microbiological communities that mediate them and the results show that microbial Fe-oxidation is a widespread process in the deep-sea environments. The apparent role that these organisms involved in iron cycling play in promoting rock and mineral weathering and precipitation is still sparsely understood and only few isolates have yet been cultivated. As a consequence of this only limited knowledge exist about iron oxidation as an ancient metabolic pathway. Therefore studying the process by which iron is oxidised and how this influence cold deep sea community is of significant importance.



During summer 2009 a research cruise was set up to study the microbial ecology of actively venting sulfide deposits along the Eastern Lau Spreading Center in South Pacific. As part of this cruise low temperature iron hydroxide deposits were sampled closed to Vai Lili and Mariner vent site (See figure right). More information about the cruise can be found at http://laugeomicro.blogspot.com/2009/06/research-objectives_12.html

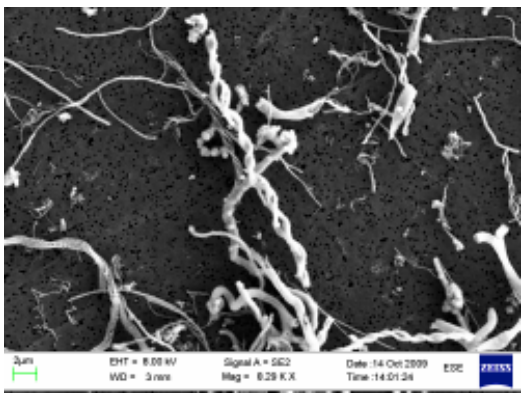



During the cruise microbial mat samples were collected at Vai Lili and Mariner. Microbial mats are dense accumulations of microbes, so thick that they resemble a mat. Samples were taken with a sucking devise (slurper). In the photo below, we were measuring the temperature at the bottom of the yellow mat, which was 48˚C and hold a Ph of 7.5.



Aims

The aim of the master project is to enrich for neutrophilic FeOB from iron oxide hydroxy deposits from hydrothermal systems in the Back Arc system and to characterise these enrichments using molecular methods to gain exciting new valuable insights into the composition, evolution, function and physiology of the heart of these “earth driven ecosystems”. In a previous master thesis work Torill Johannessen established a cultivation approach for neutrophilic iron oxidisers using a biphasic media, and she also designed new specific primers for *Mariprofundus sp.* like organisms present in hydroxide deposits from the Arctic vent site. Preliminary



data from the Lau basin samples suggest that there are several different types of potential iron oxidisers based on the characteristic structure resembling these organisms such as twisted stalks and hollow structures (See figures above).

The aims of the proposed master thesis are:

1) Enrich for iron oxidising bacteria (IOB) at neutral pH by using biphase media and incubate at 15 – 48 °C
2) Analyse the bacterial community in the enrichments using PCR and cloning approach
3) Identify microbes involved in iron oxidation in this environment

Working plan

1) Samples were collected summer 2009 by Lise Øvreås during the NSF funded cruise led By Professor Anna-Louise Reysenbach. Microbial mats has been sampled and processed for later cultivation experiments and also material has been frozen immediately for DNA extraction and molecular analyses.
2) Set up enrichments for iron oxidising bacteria using a biphasic media
3) Follow the enrichment by PCR-DGGE fingerprinting (PCR-polymerase chain reaction; DGGE-denaturing gradient gel electrophoresis) of the different samples
    a) PCR amplify 16S rRNA gene fragments with specific primers for Bacteria and IOB
    b) DGGE separation of the products
    c) Punch out and sequence selected bands that are dominant/unique
    d) Compare with sequences in databases to assess the phylogenetic affiliation of putative dominating populations
    e) Look for sequences similar to known IOB
4) Construct a PCR based 16S rRNA clone library by TOPO-TA cloning (Invitrogen) from the enrichment where potential novel IOB are found
5) Sequence some of the clones from each library and compare with sequences in the database and other well known IOB
6) Use the sequence information obtained to design specific primers/probes for the IOB present in the native samples
7) Specific PCR amplification (quantitative PCR) for IOB

Financing

The thesis will be linked to the research group in Geomicrobiology and Centre for Geobiology. We will also apply for additional funding through Bergen Forskningsttiftelse.

Working place

Geomicrobiology research group, Center for Geomibiology and Department of Biology, Marineholmen.