A novel *in situ* detection technique of metabolic enzymes in Black Sea methane seep microbial mats

C. WREDE[1]*, C. HELLER[2], J. REITNER[2] AND M. HOPPERT[1]

[1]Institute of Microbiology and Genetics, University of Goettingen, Grisebachstr. 8, 37077 Goettingen, Germany (*correspondence: cwrede@gwdg.de, mhopper@gwdg.de)
[2]Geoscience Centre, University of Goettingen, Goldschmidtstr. 3, 37077 Goettingen, Germany (cheller1@gwdg.de, jreitne@gwdg.de)

Microbial biofilms in natural habitats may contain a vast number of microorganisms with different metabolic features. Phylogenetic analysis and environmental genomics give insight into the various metabolic types present in biofilm consortia, but do not allow an assignment of expressed metabolic enzymes and hence metabolic pathways to certain microbial cells. With fluorescence *in situ* hybridization it is possible to locate organisms of distinct phylogenetic groups in biofilms by light microscopy, but metabolic key processes conducted by the organisms remain undiscovered.

To overcome this drawback, we established a procedure for generation of probes, specific for metabolic enzymes in environmental biofilms, applicable for light as well as electron microscopy. For this purpose, degenerate primers were calculated for genes from protein families of interest. Primers were used for amplification of these gene sequences from biofilm DNA. The sequences were cloned in an overexpression vector. The protein was heterologously expressed in a host strain (like *Escherichia coli*). The purified protein was used for raising polyclonal antibodies in rabbits. Correlative light/electron microscopy [1] based on the antibody probe in combination with different markers was used for loacalization of the target proteins and, by this way, the metabolic feature connected to this protein, below the scale of a single cell.

We used this method in particular to study microbial mats from deep sea methane seeps located on the Black Sea Crimean shelf. The key players of the anaerobic oxidation of methane (AOM), anarobically methane oxidizing archaea (ANME) as well as the sulfate-reducing bacteria (SRB) were analyzed by detection of the enzymes methyl-coenzyme M reductase (MCR) [2, 3] for (reverse) methanogens and the adenylylsulfate reductase for SRB.

[1] Wrede *et al*. (2008) *J. Microbiol. Methods* **73**, 85–91. [2] Heller *et al*. (2008) *Geomicrobiol. J.* **25**, 149–156. [3] Krüger *et al*. (2003) *Nature* **426**, 878–881.

Investigations of the influence of microbial cells on phosphate mineral precipitation

KAREN WRIGHT[1], YOSHIKO FUJITA[1], JAMES HENRIKSEN[1], NIC SPYCHER[2] AND MARK CONRAD[2]

[1]Idaho National Laboratory (Karen.Wright@inl.gov, Yoshiko.Fujita@inl.gov, James.Henriksen@inl.gov)
[2]Lawrence Berkeley National Laboratory (Nspycher@lbl.gov, Msconrad@lbl.gov)

The U. S. Department of Energy (DOE) is evaluating induced precipitation strategies to immobilize trace metals and radionuclides in the subsurface. One promising class of approaches is based on promoting the formation of phosphate minerals into which contaminants can partition. Phosphate minerals are relatively insoluble under conditions typical of many DOE sites, and thus contaminant sequestration within such minerals would be expected to be a long-term remediation strategy at these sites.

A potential means of promoting subsurface mineral precipitation is stimulating microbial activity that releases phosphate ions from P-containing substrates. We have been investigating whether indigenous subsurface microorganisms can facilitate mineral precipitation by degradation of the compound triethyl phosphate (TEP). A mixed microbial culture derived from Idaho National Laboratory sediments was found to be capable of degrading TEP, and releasing soluble phosphate. However, mineral precipitation appears to be inhibited despite oversaturation with respect to minerals such as hydroxyapatite and octacalcium phosphate.

To examine how microbial cells might impact calcium phosphate mineral precipitation, apart from the direct release of phosphate from TEP, we also conducted some abiotic experiments in which phosphate was added directly to the same synthetic groundwater as used for the TEP biodegradation experiments, in the presence and absence of *Comamonas testosteroni* cells. We also looked at the effect of adding organic acids that were detected in the TEP degrading cultures. Experiments where the phosphate concentration was increased incrementally over time (as would occur in the TEP degradation experiments) were conducted, as well as experiments where phosphate was added just at the beginning of the experiment. Results indicate that even in the absence of microbial cells, phosphate mineral precipitation in the synthetic groundwater is slow, but it is even more retarded by the presence of cells, as well as organic acids. The mineral phases formed initially are amorphous, but eventually appear to become more like hydroxyapatite.