ANALYSIS OF MICROBIAL COMMUNITIES DURING IRON AND SULFATE REDUCTION IN A CONTAMINATED FLOODPLAIN SOIL AT DIFFERENT TEMPERATURES

Purpose of the visit

The purpose for visiting the Geomicrobiology Group of Professor Kappler at the University of Tübingen was to characterize the microbial community of a contaminated floodplain soil during iron- and sulfate reduction by using methods from molecular biology. We planned to study i) changes in the community at different stages of soil reduction (oxic, iron reducing and sulfate reducing) ii) changes in the community at different temperatures (23°C, 14°C and 5°C) iii) differences in porewater and soil microbial community and iv) which bacteria form Cu(0) on their surface.

Work carried out

The first step was to extract the DNA from soil and filter samples. Soil DNA was extracted from 7 samples according to Zhou et al. 1996, modified. The DNA extract had to be purified by separating it from the simultaneously extracted humic substances on a 1% agarose gel. The cut gel piece was subsequently purified with a QIAEX II Gel Extraction Kit. Filter samples were extracted with a MoBio Power Soil® DNA Isolation Kit. Specific regions of the bacterial 16S rDNA fragments were amplified by PCR (polymerase chain reaction) using the primers 341F-GC and 907R and the DNA polymerase GoTaq® for subsequent DGGE (denaturing gradient gel electrophoresis). For some samples DNA purification had to be repeated several times to get an amplicon for DGGE. Concentrations of DNA extracts and PCR products were measured with a NanoDrop spectrophotometer. DGGE was performed on acrylamide gels (6%) containing a 35% to 60% gradient of denaturant (urea and formamide) in direction of electrophoresis and was run at 60°C in a 1x TAE buffer for 16h at 100V. Three DGGE gels had to be run to get good results for all soil and filter samples. They were stained with silver and photographed on a UV transilluminator. Two more DGGE gels were run for staining with Ethidiumbromide and cutting of bands that were used for cloning. The DNA was extracted from the gel pieces and used as template for reamplification PCR performed with the primers 341F and 907R which all showed positive results. The most prominent bands from two filter samples were chosen for first sequencing. Therefore the DNA from the selected bands was purified on 1% agarose gels and handled with a Promega Kit for purification, followed by cloning with competent cells of *Escherichia coli* with the TOPO® Cloning Kit. *E. coli* cells were cultured in LB solid medium containing 100 µg/mL Ampicillin and X-

gal. Transformants were selected by means of blue–white selection. White colonies were picked and screened for inserts by performing colony PCR with the primers M13F and M13R. Plasmid DNA was prepared from the clones with a Miniprep Kit and was sent for sequencing to a company.

Main results

Figure 1 shows one of the DGGE gels from the first part of this study. One set of filter samples can be seen on the left and the soil samples on the right in between the marker lanes (m). The bands that were sampled and processed for sequencing are indicated by yellow arrows, but all bands visible by staining with Ethidiumbromide were cut and frozen at -20°C. At first, only a few bands of two filter samples were selected to focus on the main microorganisms.

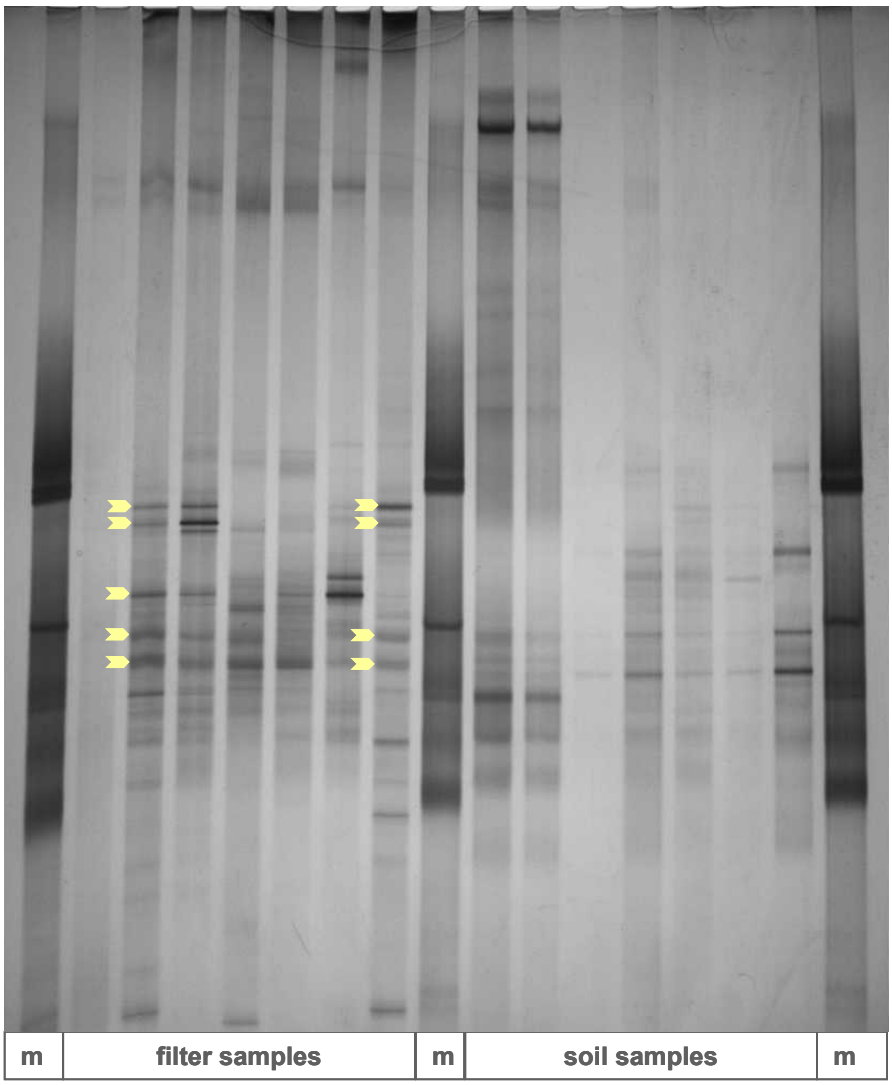

Figure 1: Silver stained DGGE gel. Marker lanes (m), filter samples (left) and soil samples (right). Yellow arrows indicate the bands that were cut and further processed for sequencing. Sample order of filter and soil samples (from left to right): 23°C: 0d, 4d, 6d, 26d; 14°C: 26d; 5°C: 34d, and 23°C: 4d different soil. (d=days).

The filter samples indicate a clear shift in the dominant bands over ~30 days of flooding at 23°C and the 26d sample resembles the 14°C sample. At 5°C a prominent band is coming up after 34 days of flooding. This gel will be analyzed with GelCompar II and compared to database entries, so that we will learn more about the structure of the community. By the time we receive the results from sequencing, they will be analyzed and the microorganisms will be identified. Further steps will be discussed afterwards.

Future collaboration

Certainly we will continue the collaboration with Prof. Kapplers group. We obtained numerous results within the 4 weeks, but there are still many open questions. The next steps will be the analysis of the sequencing results and the DGGE cluster analysis. Also the microscopy has to be carried out for total cell counts, bacteria and archaea counts. In addition, we have to identify the microorganisms forming Cu(0) on the surface by Fluorescence In-Situ Hybridization (FISH) with rRNA-targeted oligonucleotide probes. If the sequencing reveals bacteria for which no probes have been developed so far, we will have to develop the probes first. In the end we expect to identify the microorganisms bearing Cu(0) on their surface by this technique.

Projected publication

Probably the results of this study will be part of a publication projected about naturally formed colloids during iron and sulfate reduction in a contaminated soil.

Literature

Zhou, J.; Bruns, M.A.; Tiedje, J.M.; DNA recovery from soils of diverse composition. Appl. Environ. Microbiol. 1996, 62, 316–322.