

## *In situ* analysis of the spatial distribution of methanogenic *Archaea* in the anoxic sediment of Lake Rotsee

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Anoxic sediments and soils are the natural habitat for the strict anaerobic methanogenic *Archaea*, which can be detected ubiquitously. Methanogens play a significant role in carbon mineralization, nutrient recycling and the emission of the trace gas CH<sub>4</sub>. The current study aimed to characterize the dynamics of methanogens in the anoxic sediment of lake Rotsee. Therefore *in situ* hybridization was used to quantify the spatial distribution of methanogens, besides total *Bacteria* and *Archaea*, throughout the upper 10 cm of a sediment core. The results were compared with the methane profile from corresponding depths.

### Methods

At the sampling site lake Rotsee, Switzerland, a sediment core and a preincubated dialysis pore-water sampler ('peeper' (1)) were gathered in the beginning of September. Methane concentrations in the pore-water were measured by head-space gaschromatography. The sediment core was extruded and sampled in 1 cm intervals, fixed immediately with 96% ethanol and stored at -20°C until analysed by *in situ* hybridization. In addition, nucleic acids were extracted from the top layer of the core and investigated to retrieve *Archaea* specific 16S rRNA sequences (2). The 16S rRNA sequences were compared with EMBL Nucleotide Sequence database by FASTA analysis, using the GCG Wisconsin package (3). Subsequently the sediment retrieved sequences were aligned, phylogenetically analysed by PileUp and investigated for specific probe design with the ARB probe design program (Dr. W. Ludwig, Technical University of Munich, Germany). Finally two oligonucleotide probes were constructed, which target about 50% of the sediment retrieved

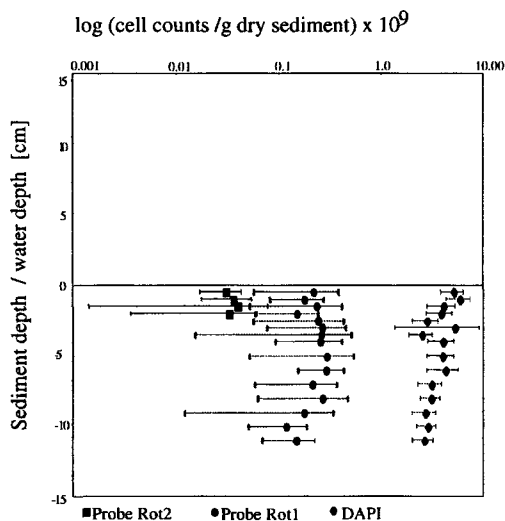
sequences, and named probe Rot1 and Rot2, respectively. Subsequently, *in situ* hybridization analysis of the sediment material was performed by the investigation of Cy3-labeled fluorescent oligonucleotide probes Eub338 (4, 5), Arch915 (6) Rot1 and Rot2. In addition, total cell count was determined using the fluorescent dye DAPI.

### Results and discussion

After PCR-assisted sequence retrieval, cloning and sequencing, the phylogenetic analysis resulted in the identification of two main clusters of sequences. The sequences grouped either with members of the genus *Methanosaeta* or clustered with *Plagiopyla nasuta* endosymbiont 16S rDNA. Against these two clusters the oligonucleotide probes Rot1 (*Methanosaeta*) and Rot2 (*P. nasuta* endosymbiont 16S rDNA) were designed and used for *in situ* hybridization. Results of cell counts are shown in Fig. 1, together with methane concentrations of corresponding depths in Fig. 2. Highest population densities of methanogens were found in the surface sediment layers. This confirmed the results from methane profiles and activity measurements which indicated highest methanogenic activity in these layers (not shown). Moreover, probe Rot1 identified the majority of *Archaea* present throughout the whole core, whereas probe Rot2 characterized a methanogenic population that could be exclusively detected in the upper two centimeters of the core.

### Conclusion

The here investigated molecular tools proved capable to analyse the methanogenic community of an anoxic

FIG. 1. *In situ* hybridization counts.

sediment. Population densities decreased with depth and correspond with the methane profile. In addition, the differences in community structure confirmed activity measurements with different substrates. This indicates the dependence of community composition from the availability of certain substrates which needs further investigation.

### References

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### CH<sub>4</sub> in porewater [mM]

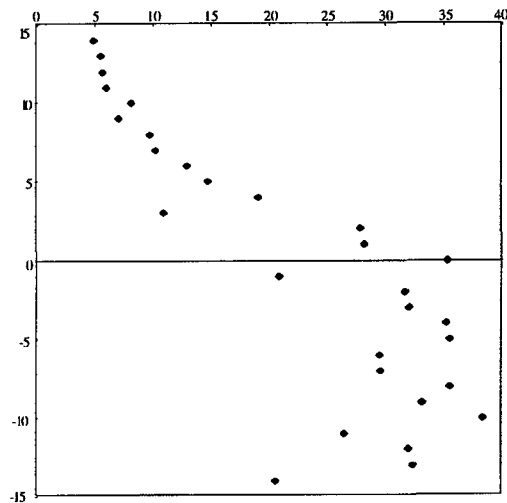


FIG. 2. Methane profile.

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