

RNA – A Tracer to Detect Microorganisms

The group “Molecular Ecology” at EAWAG in Kastanienbaum uses 16S rRNA, a structural element of ribosomes, as a tracer in studies on the carbon cycle in anaerobic sediments. Two examples are presented here. Fluorescence *in situ* hybridization was used to identify active bacteria in 150 year old sediments from Baldeggersee. Two populations of methane-forming *Archaea* were characterized in Rotsee sediments; their depth distribution was correlated with the quality of available organic material.

RNA as a Molecular Tracer

According to recent estimates, less than 1% of the naturally occurring prokaryotes (bacteria within the domains *Bacteria* and *Archaea*) are identifiable by traditional culture methods. The recent development of molecular tracers, however, allows us to confirm the presence of microorganisms in the environment that have not yet been cultured in the laboratory.

Ribonucleic acid (RNA) can be classified as mRNA, tRNA, or rRNA, according to its specific structure and function. When there is

need for a particular protein, mRNA (messenger RNA) transports the genetic information in the form of a copy to the ribosomes where protein synthesis takes place. The tRNA (transfer RNA) brings the necessary amino acids to the ribosome where they are linked together to proteins. Ribosomes themselves are made up of rRNA subunits (ribosomal RNA) of different size (i.e., 5S, 16S and 23S rRNA; S = Svedberg unit, representing the velocity at which suspended biomolecules sedimentate under standard centrifugation conditions).

16S rRNA (Fig. 1) is a particularly useful tracer in molecular ecology [1]. Comparative analyses of the molecular sequences of 16S rRNA isolated from a range of prokaryotes have shown that it possesses both highly conserved and highly variable regions [2]. Conserved regions, for example, show the same sequence for all prokaryotes, while highly variable regions can help to identify specific groups of prokaryotes. Based on these differences, it is possible to design RNA probes with different selectivity. RNA probes contain approximately 20 nucleotides that are complementary to the selected target region on the rRNA. An enormous number of gene probes for the selective identification of a wide range of groups of prokaryotes have been published to date [3]. Some examples are given in Fig. 1. Using fluorescence *in situ* hybridization [4], a given RNA probe is labeled with a fluorescent dye and mixed with an environmental sample in high concentrations. The probe diffuses into the prokaryotic cells and binds to (hybridizes with) its complementary target on the rRNA within the prokaryotic cell without destroying its structure. Marked cells are visible under a fluorescence microscope and can be quantified by direct counting (Fig. 2).

Active Bacteria in 150 Year Old Sediments

We used fluorescence *in situ* hybridization to identify metabolically active prokaryotes



Fig. 2: *In situ* hybridization of a sediment sample with the RNA probe Arch915 detecting *Archaea* [5] as indicated by arrows.

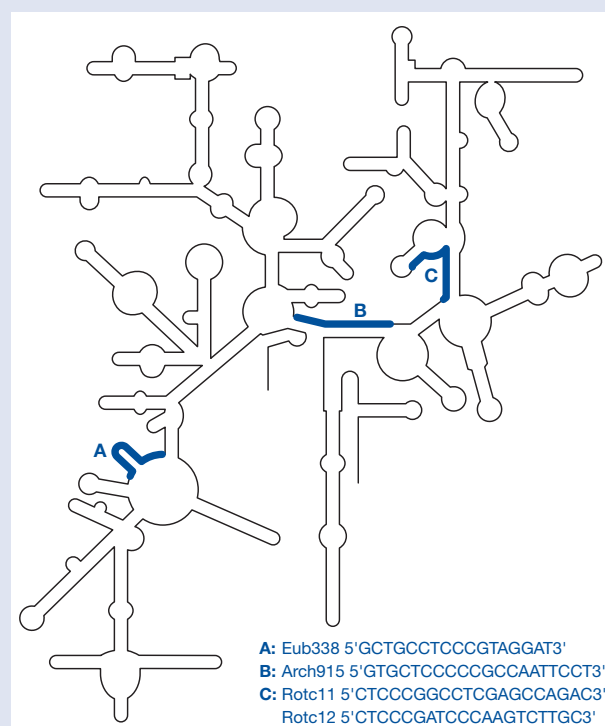


Fig. 1: Two-dimensional structure of 16S rRNA of *Escherichia coli* with conservative sections (A and B) and a variable section (C) which bind different genetic organisms. Eub338 identifies organisms in the domain *Bacteria*; Arch915 detects organisms in the domain *Archaea*; the gene probes Rotc11 and Rotc12 identify two different populations of methanogenic *Archaea*.



in a sediment core from Baldeggersee. Among others, we used the probe Eub338 [5], which detects all organisms of the domain *Bacteria* (Fig. 1). The density of bacteria was highest in the first few centimeters and then decreased with depth. Layers below 40 cm are older than 150 years and showed only minimal cell counts (Fig. 3). Using culturing techniques, however, we were able to confirm the presence of active microorganisms in these sediments, which are capable of completely degrading organic material in sediment layers that were as old as 6000 years.

Methanogens in Rotsee Sediments

In a second study, we investigated which specific methanogens are responsible for the anaerobic degradation of organic material in the sediments of Rotsee [6]. The first task was to find RNA probes that were selective for the methanogens present in this site. We extracted the total nucleic acid fraction from the top 10 cm of a sediment core. This fraction served as a template for a selective polymerase chain reaction (PCR) in which the 16S rRNA genes of the indigenous methanogens present in the studied sediment core were amplified [7]. Subsequent sequencing of the PCR fragments allowed us to synthesize two specific probes: Rotc11 identifies a group of methanogenic *Archaea* that form methane (CH_4) from CO_2 and H_2 , while Rotc12 detects methanogens that transform acetate to CH_4 and CO_2 (Fig. 1).

These two probes Rotc11 and Rotc12 were used in fluorescence *in situ* hybridization assays to determine the depth profile of the

two populations (Fig. 4). Results showed that the acetate-using *Archaea* were present at all depths, while the methanogens transforming CO_2 and H_2 to methane were restricted to the top two centimeters of the sediment. These bacteria appear to require freshly sedimented organic material.

Our Goals

The two projects described here demonstrate the versatility of methods using rRNA as a tracer. The group "Molecular Ecology" at EAWAG Kastanienbaum is using these methods, combined with chemical analyses, in an interdisciplinary effort to comprehensively characterize microbial communities in natural aquatic systems.



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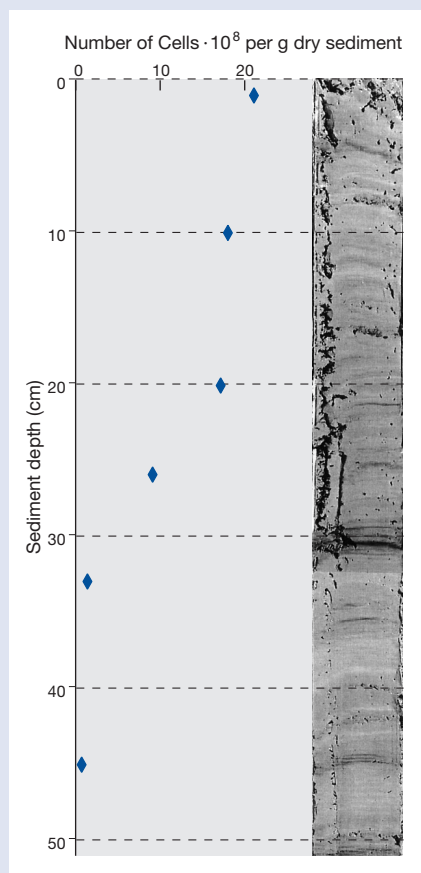


Fig. 3: Cell counts in the top 50 cm of a sediment core from Lake Baldegg, Switzerland (number of cells per gram dry weight of sediment). Microorganisms were made visible by fluorescence *in situ* hybridization (FISH) and counted under a fluorescence microscope. The RNA probe used was Eub338.

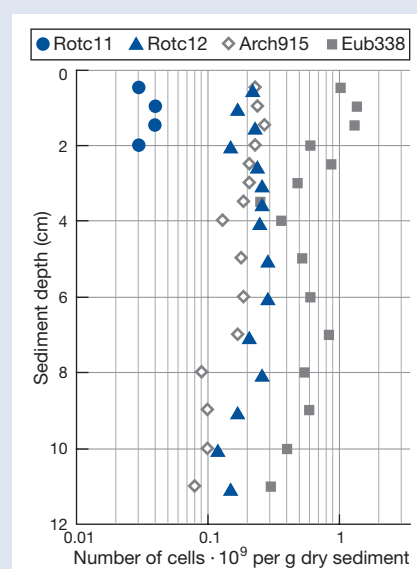


Fig. 4: Distribution of different groups of microorganisms in Rotsee sediment (per g dry wt). Identification with fluorescence *in situ* hybridization using various gene probes.

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