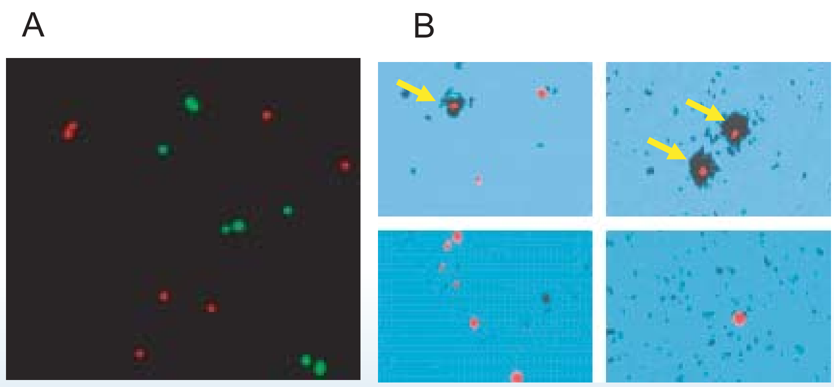
MAJOR ROLE OF ARCHAEA IN THE DEEP NORTH ATLANTIC OCEAN

*Eva Teira, Thomas Reinthaler & Gerhard J. Herndl**

Archaea form one of the three branches of the Tree of Life. The other two are the Bacteria and the Eukarya; the latter include all plants and animals. Archaea have been traditionally considered as "extremophiles," although recent studies have shown that non-extremophilic Archaea are widespread in marine plankton of virtually all seas and oceans. We showed that Archaea play a major role in the ecosystems of the deep and dark parts of the North Atlantic and found indications for growth on inorganic as well as organic carbon. Thus they might have an autotrophic and/or a heterotrophic mode of growth.

Despite the general occurrence of Archaea, virtually nothing is known yet about their basic physiology. Questions as: “What are their sources for carbon and energy?” need to be answered to assess the role of Archaea in the global biogeochemical cycles. We have adapted a molecular technique (CARD-FISH, see the intermezzo for a more detailed explanation) to detect marine Archaea more efficiently. This technique was applied to samples collected during 2 research cruises in the North Atlantic where the two main branches of the North Atlantic Deep Water were followed over more than 4000 km. Water samples were collected from 100 to 4000 m depth. By using MICRO-CARD-FISH, we determined the substrate uptake on a single cell level for Bacteria, Eury- and Crenarchaeota separately. As substrate we used radiolabeled bicarbonate to indicate potential autotrophy of Archaea and Bacteria and enantiomeric amino acids. Our results indicate that Archaea are not only more abundant than Bacteria but provide also direct evidence that Archaea are, at least partially, living in an autotrophic mode utilizing inorganic carbon in a similar manner as plants do, but without sunlight as energy source. However, Archaea are also taking up organic molecules like amino acids, indicating heterotrophic growth. Whether individual archaeal species are capable to grow on both inorganic and organic carbon sources and the source they use for energy, are both very important research questions for the near future. NIOZ is very well equipped to play a leading role in this field.



Examples of applying CARD-FISH and MICRO-CARD-FISH to North Atlantic deep water prokaryotic communities (2700 m). (A) Confocal laser scanning micrograph of a double CARD-FISH hybridisation. Bacteria fluoresce green and Archaea fluoresce red. (B) Micrograph of Archaea assayed by MICRO-CARD-FISH using radio-labelled Aspartic acid. Active cells surrounded by black halos are indicated by yellow arrows.

*Corresponding author: herndl@nioz.nl

What is ‘FISH’?.

The combination of Fluorescence In Situ Hybridisation (FISH) techniques with microautoradiography allows us to determine the uptake potential of a given substrate by specific prokaryotic groups (Bacteria or Archaea).

The recently developed Catalyzed Reporter Deposition (CARD)-FISH uses horseradish peroxidase (HRP)-labelled oligonucleotide probes and is based on the deposition of a large number of fluorescent molecules by peroxidase activity, resulting in the amplification of the fluorescence signal. The critical step of this method is the penetration of the HRP-labelled oligonucleotide probes into the cells, due to the large size of the enzyme. The original protocol included a lysozyme permeabilisation treatment. However, Archaea cell walls do not contain murein, and thus, they are insensitive to lysozyme treatment. We therefore modified the existing protocol to more efficiently detect marine Archaea, substituting lysozyme by proteinase-K treatment to make the cell wall permeable for the probe. The adapted method with proteinase-K resulted in detection rates of Archaea which were twice as high as with lysozyme pre-treatment. We successfully combined micro-autoradiography and the refined CARD-FISH (MICRO-CARD-FISH) method for the study of the uptake of specific substrates by Bacteria and Archaea in the deep waters of the North Atlantic (from 100 down to 4000 m depth).