MICROAUTORRADIOGRAPHY AND CATALYZED REPORTER DEPOSITION FLUORESCENCE *IN SITU* HYBRIDIZATION (MICRO-CARD-FISH) FOR STRUCTURE-FUNCION ANALYSIS OF PROKARYOTES IN DEEP WATERS.

Eva Teira * and Gerhard J. Herndl

Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research, P.O. Box 59, 1790 AB, Den Burg, The Netherlands
* teira@nioz.nl

Abstract

Only recently, fluorescence in situ hybridization (FISH) became sufficiently sensitive to allow the enumeration of prokaryotes even in oligotrophic waters by using catalyzed reporter deposition (CARD-FISH). Penetration of the horseradish peroxidase (HRP)-labeled oligonucleotide probes into prokaryotic cells needs careful adaptation to the target microoganisms. We refined the CARD-FISH protocol for detection of marine Archaea substituting lysozyme by proteinase-K as permeabilization treatment. Detection rates of Archaea with proteinase-K (65±6 % of total DAPI counts) were twice higher than with lysozyme (32±6%). Combining CARD-FISH with microautoradiography, we determined the uptake of specific organic compounds by the major prokaryotic groups in deep water samples.

Keywords: FISH, microautoradiography, Bacteria, Archaea, deep ocean.

Introduction

Over the last decade, our knowledge on the richness of marine prokayotic communities including the deep ocean has increased considerably through the application of molecular tools as fingerprinting techniques, cloning and sequencing. An exciting result derived from the application of these molecular approaches was the discovery of the widespread presence of archaea in marine plankton (1, 2). However the assesment of the actual abundance of specific groups or populations of prokaryotes is more difficult. Basically the only direct method available is the fluorescence *in situ* hybridization (FISH), which yielded very low recovery of Bacteria and Archaea as compare to nucleic acid-stained in oligotrophic environments.

Only recently, the use of polynucleotide probes allowed the assessment of the abundance of Archaea in the meso- and bathypelagic waters of the Pacific (3). Unfourtunately, to tha date, the use of oligonucleotide FISH has not been succesfully applied in deep waters. The recently developed Catalyzed Reporter Deposition FISH (CARD-FISH) for the identification of marine bacteria (4) allows the use oligonucleotide probes labeled with horseradish peroxidase (HRP) with great recover efficiency. However, this protocol included a permeabilisation procedure with lysozyme. Archaeal cell walls do not contain murein (5), thus, Archaeal cells are not sensitive to lysozyme. In this study, we modified the CARD-FISH for detection of marine Archaea substituting the lysozime permeabilization treatment by proteinase-K treatment and we succefully combined CARD-FISH with Microautorradiography (MICRO-CARD-FISH) for the detection of uptake of specific organic compound by deep-water prokaryotic communities.

Results and discussion

We selected a total of 10 samples from deep waters (100-2750m) of the North Atlantic Ocean for comparison of permeabilisation procedures, tus covering a wide range of Archaeal abundance. The percentages of DAPI counts detected with specific Archaeal probes (Eury for Euryarchaeota; and Cren for Creanarcaeota) were considerably higher with proteinase-K than with lysozyme pre-treatment (Fig 1). The percentage of DAPI cells detected with Eury probe was 14 ± 2% (±SE, n=10) with lysozyme and 30 ± 2% (± SE, n =10) with proteinase K. Similarly, the rate of detection with Cren probe was 18 ± 2% (±SE, n=10) with lysozyme and 35 ± 5% (±SE, n=10) with proteinase-K. Overall, we were able to double our capacity for Archaeal detection by modifying the permeabilisation procedure.

We further combined microautorradiography with the refined CARD-FISH for detection of prokaryotic activity in meso- and bathypelagic waters. We used tritiated D- and L-Aspatic acid (Asp) as substrate, and three different probes for the major prokaryote groups: EUB for bacteria, and the two Archaeal probes described above (Eury and Cren)(Fig. 2). The percentage of active cells decreased with depth for the L-Asp both for bacteria and Archaea. However, the D-Asp showed contrasting uptake patterns between bacteria and Archaea. Whereas the percentage of active bacteria decreased with depth, the percentage of active Archaea increased from subsurface waters (100 m) to bathypelagic waters (1000-3000 m). Whereas in subsurface waters bacteria and Archaea seem to be qually active, in deeper water, a higher proportion of Archaea is actively taking up D- or L-Asp.

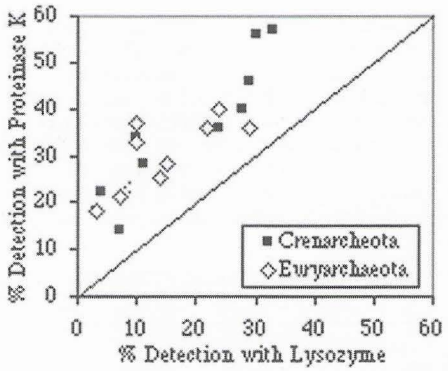

Fig. 1. Comparison of the detection rates (percentage of probe-hybridized cells normalized to total DAPI-stained cells) by CARD-FISH for Crenarchaeota and Euryarchaeota using lysozyme and proteinase-K treatment for cell wall permeabilization.

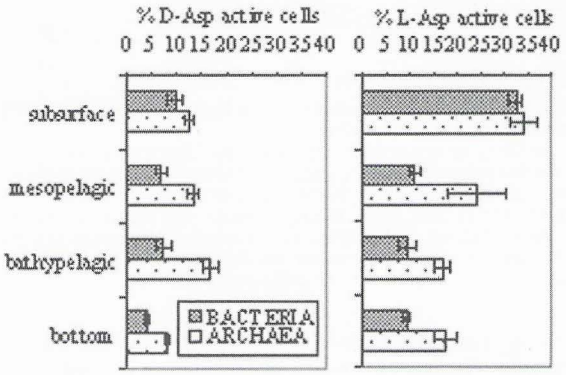

Fig. 2. Percentage of Bacteria, Eury- and Crenarchaea taking up D-and L-Asp normalized to the total number of each group, in different depth layers of the North Atlantic. Bars represent the mean (± SE) from 10 different stations.

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