

# Microbial Diversity and Activities within the Subsurface of Tidal Flat Sediments

Bert Engelen, Henrik Sass<sup>1</sup>, Beate Köpke, Reinhard Wilms, and Heribert Cypionka

Institut für Chemie und Biologie des Meeres, Universität Oldenburg, Postfach 2503, D-26111 Oldenburg ([www.icbm.de/pmbio](http://www.icbm.de/pmbio))

<sup>1</sup> Present Address: School of Earth, Ocean and Planetary Sciences, Cardiff Uni, Main Building, Park Place, Cardiff, CF10 3YE, Wales - UK

## Introduction



Fig. 1: Sampling sites at the German Wadden Sea near the island of Spiekeroog

A combination of geochemical, microbiological and molecular biological techniques was applied to analyse the subsurface of tidal flat sediments. Cores were taken at two sampling sites down to a depth of 5.5 m (Figs. 1 & 2). DGGE analysis revealed that the upper layers were dominated by various Proteobacteria. Below this zone, a dramatic shift to the Chloroflexus group was observed (Figs. 4 & 6). A slightly different shift was also detected by the cultivation approach (Fig. 5). In total, 112 isolates could be grouped into 53 different OTUs. In deeper sediment layers almost exclusively endospore-forming bacteria were detected. To link the information of who is there with geochemical profiles, exoenzyme activities and sulfate reduction rates were measured. The abundance of sulfate reducers was determined by quantitative PCR (Fig. 3).



Fig. 2: The sediment cores were taken by a vibrocorer and recovered by a lifting block.

## Results

- The lithological characterization identified three major zones. An upper part which is dominated by sand followed by a shell bed and a muddy zone in deeper layers.
- A sulfate peak within the shell bed might be caused by a lateral inflow from an adjacent tidal creek.
- The amount of *dsrA* genes was high in layers where sulfate was still present. The curve follows the sulfate profile.

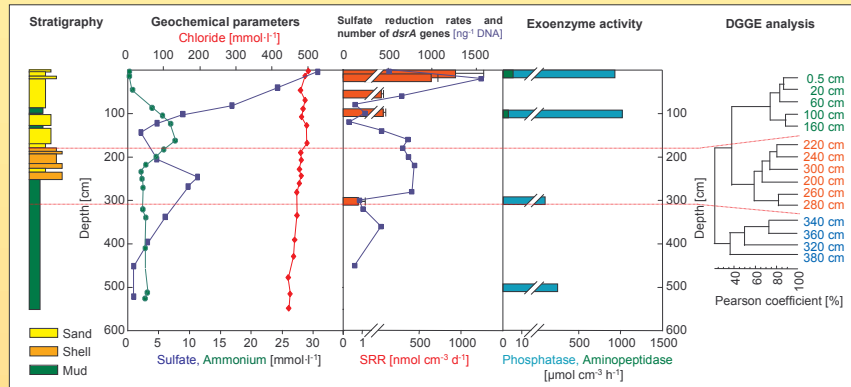


Fig. 3: Geochemical, microbiological and molecular biological parameters determined along the sediment column of site Neuharlingersiel Nacken.

- The highest sulfate reduction rates were determined in the upper layers, but sulfate reduction was still detectable in 300 cm.
- Phosphatase activity showed high values down to 550 cm. The Activity of aminopeptidase was detectable down to 100 cm, only.
- The DGGE analysis showed three clusters of microbial community composition that corresponded to the three main stratigraphic zones.

### Molecular biological investigation

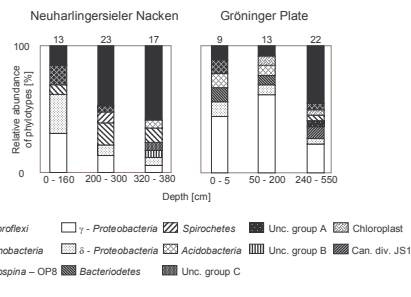


Fig. 4: Cumulative plot of bacterial phyla detected by DGGE. The composition was calculated by the number of phylotypes including bands with corresponding positions.

- In the molecular biological approach (Fig. 4), most of the identified DGGE bands affiliated with so far uncultivated phyla.
- The amount of so far uncultivated phylotypes increased with depth and were dominated by Chloroflexi (Figs. 4 & 6).
- In the cultivation approach (Fig. 5), a shift to spore-forming microorganisms with depth was observed.
- Several strains will be described as new genera: Strains N05VI, N05VIII closest relative *Cytophaga fermentans*, Bacteroidetes (87%), strains N05VII, N5V, N50X closest relative *Ilyobacter tartaricus*, Fusobacteria (93%), strains N5VI, N100XI closest relative *Eubacterium angustum*, Firmicutes (91%).

### Microbiological investigation

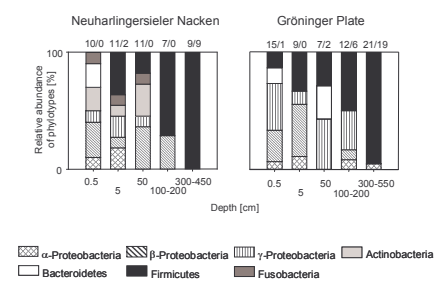


Fig. 5: Relative contribution of the different phylogenetic groups to the culture collection. The numbers of isolates and spore-forming strains are given.

- The *Chloroflexi* represent a widespread phylogenetic group that is found in subsurface environments, but also in soil, wastewater, geothermal, and polluted sites. All of the detected *Chloroflexi* sequences fall in subdivision two that contains only sequences from a subsurface origin.

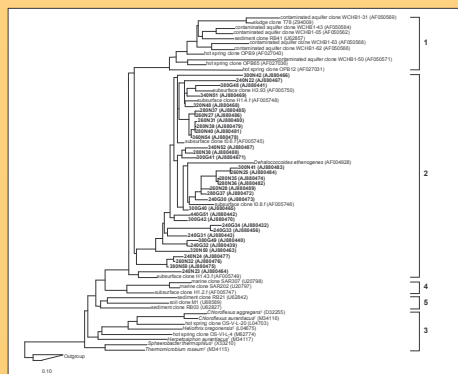


Fig. 6: Phylogenetic tree of the Chloroflexi. The origin of each environmental sequence is indicated. Wadden Sea sequences are given in bold and are marked by N for site Neuharlingersiel Nacken and G for site Gröninger Plate.

## Conclusions

- Microbial activities were detectable along the entire sediment column and were governed by the sediment composition and geochemical parameters.
- Both, the molecular biological and the microbiological investigation showed a shift in community composition with depth. On one hand to spore-forming microorganisms, on the other hand to so far uncultivated phylotypes.
- The detection of a typical deep-biosphere subphylum of the Chloroflexi raises the question at which depth and age the upper boundary of the deep biosphere is located. Thus, tidal flats might offer a convenient possibility to study and understand certain aspects of the deep biosphere in general.

### Acknowledgements

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