Microbial community composition of the “shallow biosphere” of Wadden Sea sediments

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Introduction

Fig. 1: Sampling sites at the East Frisian Wadden Sea near Spiekeroog

Microbial communities from the upper 20 cm of Wadden Sea sediments have been investigated repeatedly. However, very little is known about the sediment layers below, a zone we call the “shallow biosphere“. To fill the gap of information, a sampling campaign was performed at the backsite barrier tidalflat of the island of Spiekeroog. We have recovered up to 6 m long sediment cores from three locations to analyse microbial communities along vertical profiles. The samples were treated by geochemical, classical microbiological, and molecular biological techniques to get a general overview on the diversity and activity of the investigated microbial communities. The following results are based on the molecular biological analysis of the sediment samples.

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

Methods

- Total DNA and RNA of the first 2 m were extracted by bead-beating and phenol-chloroform-extraction.
- For deeper sediment layers we have used the FastDNA® Spin® Kit from Q-BIOgene.
- RNA-samples were checked for DNA residues via a control PCR.
- rDNA and rRNA were amplified by eubacterial primers (GC 357f, 907r) using PCR and RT-PCR, respectively.
- The amplicons were analysed by DGGE.
- DGGE analysis was performed on the electrophoresis system of Ingeny. An optimal separation was obtained by a gradient of 50 – 70 % denaturant.
- The bandpatterns were analysed by cluster analysis using GelCompar II.
- For a more detailed view on the community composition, bands were cut out, reamplified and sequenced.

Results

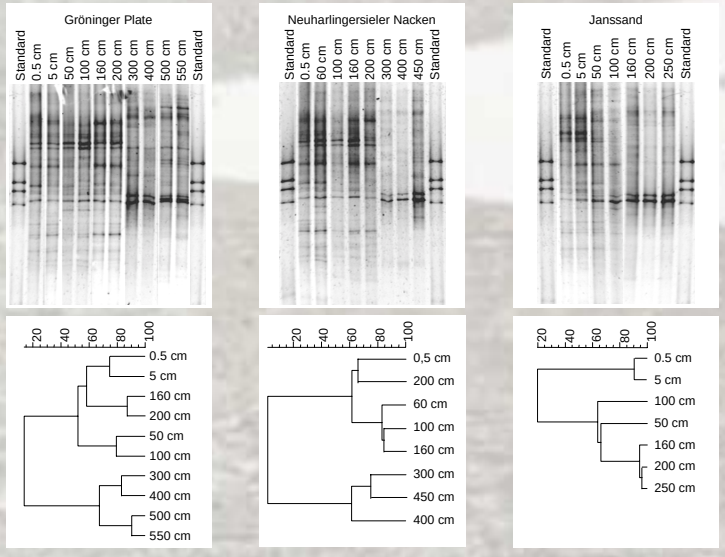

Fig. 3: Analysis of the vertical variation from three cores. The dendrograms were calculated by Pearson correlation and UPGMA.

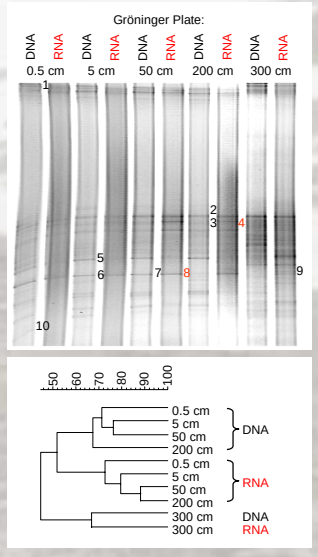

Fig. 4: Comparison of rDNA and rRNA in one core. Dendrogram calculated by Pearson correlation and UPGMA.

Phylogenetic affiliation of extracted bands from the DGGE patterns ot the Gröninger Plate :

1. *Desulfotalea psychrophila* (93 %)
2. Uncultured *Gammaproteobacterium* (97 %)
3. Uncultured *Chromaciaceae* (87 %)
4. Uncultured *Chromaciaceae* (87 %)
5. Uncultured *Deltaproteobacterium* (97 %)
6. *Thiorhodovibrio winogradskyi* (88 %)
7. *Thiorhodovibrio winogradskyi* (93 %)
8. *Thiorhodovibrio winogradskyi* (93 %)
9. Uncultured *Deltaproteobacterium* (87 %)
10. Uncultured *Holophaga/Acidobacterium* (96 %)

- Differences between the rDNA- and rRNA patterns in the upper part of the core (0 cm – 200 cm) indicate the presence of abundant but not active microorganisms. Such a discrepancy is not visible between rDNA- and rRNA patterns at 300 cm depth.
- DGGE bands with the same position in rDNA and rRNA lanes show homologous sequences (bands 3, 4 and 7, 8).

- A general dendrogram for all the investigated sediment samples showed only a separation between top- and deeper layers, because of the high similarity within the corresponding zones of the cores. Therefore, a cluster analysis of the DGGE band patterns was calculated separatly for each core.
- The structure of the single dendrograms indicate significantly different microbial communities within the top- and the deeper sediment layers at all sites.
- The clustering of samples from intermediate layers (50 cm - 200 cm) is visible, but not significant.

Conclusion and Perspectives

- The DGGE patterns indicate a dramatical decrease of microbial diversity with depth.
  - ➢ We will expand the sequencing of bands to get more information on the microorganisms, that dissappear or come up in the deeper parts of the sediment.
  - ➢ More cores will be taken from other sites of the tidal flat to proof this observation.
- The cores can be classified in three main zones: top- intermediate- and deep layers.
  - ➢ These zones will be compared between the different cores by extended sequencing of DGGE bands.
  - ➢ In future sampling campaigns we will collect more physico-chemical data to find indications on physiologically specialised microorganisms.
- The comparison between rRNA- and rDNA derived DGGE patterns seems to be appropriate to distinguish between active- and inactive microorganisms at this habitat.
  - ➢ We will validate our data with other activity markers, such as phospholipid-analysis, or enzyme activity measurements.

Acknowledgements

We thank the Senckenberg Institute, Department for Marine Research and especially the crew of the RV Senckenberg for their help to recover the sediment cores. This work was supported by a grant of the Deutsche Forschungsgemeinschaft (DFG).

For more details on the investigation of the cores by classical microbiological methods see also the poster PH036 by B. Köpke et *al*.