MICROBIAL COMMUNITY COMPOSITION OF THE "SHALLOW BIOSPHERE" OF WADDEN SEA SEDIMENTS

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Introduction

Microbial communities from the upper 20 cm of Wadden Sea sediment were investigated previously (LLOBET-BROSSA *et al.,* 1998). 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 in the backbarrier tidal flat of the island of Spiekeroog. We recovered up to 6 m long sediment cores from three locations (Neuharlingersieler Nacken, Gröninger Plate and Janssand) to analyze microbial communities along vertical profiles. To get a general overview of the diversity and activity of the microbial communities present, the samples were treated by geochemical, classical microbiological, and molecular biological techniques. The following results are based on the molecular biological analysis of the sediment cores.

Total nucleic acids (DNA and RNA) were extracted from several sample along the depth profiles of the sediment cores. To obtain "fingerprints" of the microbial communities, universal bacterial primers were used to amplify part of the 16S rDNA by polymerase chain reaction (PCR). Amplification products were analyzed by denaturing gradient gel electrophoresis (DGGE) to compare variations in community composition. The technique reproduces band patterns in an electrophoresis gel, and each band represents one sequence type indicating one bacterial species. These band patterns visualize "fingerprints" of the investigated microbial communities. To get a more detailed view of the community composition, we cut out bands of interest and identified them by 16S rDNA sequencing.

To determine the metabolically active community members, part of the extracted RNA was analyzed additionally by reverse transcription PCR (RT-PCR). In theory, metabolically active bacteria show a higher ribosome content. If RT-PCR products of the ribosomal RNA are compared with PCR products of 16S rDNA on a DGGE gel, metabolically active bacteria are indicated by a higher band intensity.

The metabolic active part of the microbial communities was exemplarily investigated for the core from the Gröninger Plate down to a depth of 3 m. Therefore, we coextracted DNA and RNA to compare 16S rDNA and 16S rRNA derived bandpatterns via DGGE and identified several abundant and active microorganism by sequencing. A comparison of the different community fingerprints was performed by cluster analysis.

Sample collection

Three sediment cores were collected in the backside barrier tidal flat of the island of Spiekeroog (Table 1). The cores were taken by a vibrocorer and cut longitudinally. For the investigations of the cores we took subcores every 20 cm beginning at a depth of 40 cm using a sterile 5 ml syringe. To obtain contamination-free samples, the subcores were taken in the middle of the main cores, a part that was not affected by coring process. To avoid air contamination, we took only the first part of the samples in the syringe for further analyses and discarded the last part, which potentially could be air contaminated.

The first 30 cm of the core were perturbed during coring. Therefore, we additionally took short cores at each location to obtain undisturbed samples from the first 30 cm. Samples were collected by taking subcores at 0 cm, 5 cm and 20 cm. The subcores were stored at -20 ℃ for further molecular biological investigation.

Extraction and amplification of nucleic acids

Genomic DNA was extracted from 1 g sediment of each subcore from the first 2 m by bead-beating and phenol-chloroform-extraction. For deeper sediment layers we used the FastDNA® SpinKit from Q-BIOgene.

RNA was coextracted with the DNA especially from the Gröninger Plate samples up to a depth of 3 m. After cell disruption by bead-beating, the samples where separated into a DNA and an RNA aliquot. The DNA was purified by phenol-chloroform-extraction. For purification of the RNA, DNase (Boehringer Mannheim) was used to digest the DNA in the RNA extract. The DNA-free RNA samples were checked for DNA residues *via* a control PCR.

To analyze the microbial composition, part of the 16S rDNA and 16S rRNA from the extracted genomic nucleic acids were amplified by eubacterial primers (GC 357f, 907r) using PCR and RT-PCR, respectively.

DGGE analysis

The PCR/RT-PCR amplicons were purified and concentrated to a volume of 10 µl by the MinElute PCR purification Kit from Qiagen. DGGE was performed by using the INGENYphorU-2 system (Ingeny). Purified PCR products were loaded onto polyacrylamide gels (6% wt/vol) in 1 x TAE (40 mM Tris, 20 mM acetate, 1 mM EDTA), with a denaturing gradient from 50 to 70% (where 100% denaturant contains 7 M urea and 40% formamide). Electrophoresis was performed at a constant voltage of 120 V and a temperature of 60℃ for 20 h. After electrophoresis, the gels were stained for 1.5 h in 1 x SybrGold solution (Molecular Probes) in 1 x TAE, washed for 30 min in distilled water, and documented by a digital imaging system (BioDocAnalyze, Biometra).

The resulting band patterns were analyzed by cluster analysis using the software package GelComparII. The dendrograms were calculated by Pearson correlation and UPGMA. DGGE bands were cut out by a sterile scalpel and reamplified via PCR. The PCR products of the reamplified bands were loaded onto a second DGGE gel to check the purity of the new amplicons.

The 16S rDNA and 16S rRNA sequences were determined by the cycle sequencing method of Sanger (SANGER *et al.,* 1977) with the LiCor DNA Sequencing System 4000 (MWG Biotech).

Comparison of the microbial communities of the three sediment cores

To analyze the depth profiles of the cores, cluster analysis of the DGGE band patterns was performed separately for each

Table 1. Locations of sampling sites.

Location	Latitude	Longitude	Absolute altitude	Depth of the core	Date of sampling
Neuharlingersieler Nacken	53°43´270 N	07°43´718 W	-58. 0 cm	5.50 m	04.06.02 at 14 pm
Gröninger Plate	53°43´638 N	07°45´960 W	-122.5 cm	4.50 m	05.06.02 at 13 pm
Janssand	53°44´495 N	07°40´415 W	-37.5 cm	2.50 m	05.06.0 2 at 15 pm

core (Fig. 1). A general dendrogram for all the investigated sediment samples from the three cores showed a clear separation between top and deeper layers and a high similarity within the corresponding zones. The structures of the single dendrograms indicated significantly different microbial communities within the top and the deeper sediment layers at all sites. A clustering of samples from intermediate layers (50 cm - 200 cm) was recognizable, but not significant.

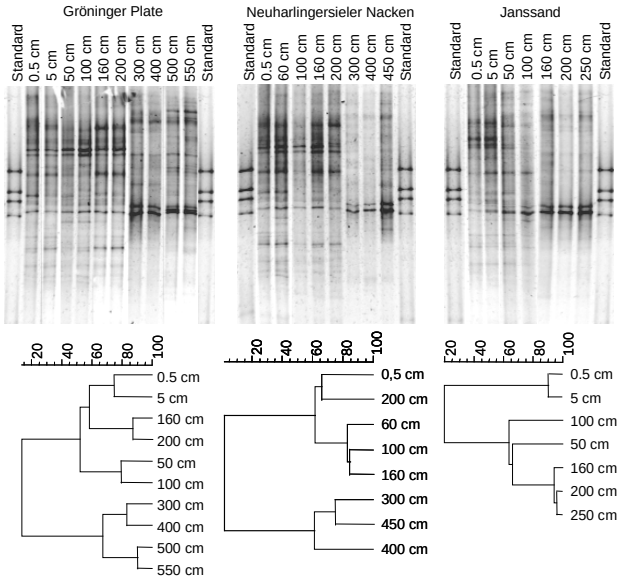

Fig. 1. Analysis of the vertical variation within three cores. The dendrograms were calculated by Pearson correlation and UPGMA.

Analysis of RNA patterns as a marker for microbial activities

The comparison between the 16S rDNA and 16S rRNA patterns in the upper part of the core from Gröninger Plate (0 cm – 300 cm) indicated the presence of a significant number of abundant but not active microorganisms (Fig. 2). Such a discrepancy between 16S rDNA and 16S rRNA patterns was not visible at a depth of 300 cm. DGGE bands with the same position in 16S rDNA and 16S rRNA lanes showed homologous sequences (bands 3, 4 and 7, 8).

Conclusions and perspectives

According to their microbial community structure, the three sediment cores can be classified into three main zones: top, intermediate and deep layers. In future analyses, we will extend sequencing of DGGE bands to define the microbial composition of the three main zones at the investigated locations. To get an impression which microorganism are abundant at the different locations, we will compare the sequences from the three cores to see differences and equivalents in the composition of the microbial communities.

The DGGE patterns of all the cores showed a dramatical decrease of microbial diversity with depth. This change in the community composition was located between 2 m and 3 m for Neuharlingersieler Nacken and Gröninger Plate and between 50 cm and 100 cm for Janssand. To locate the layer of discontinuity we will investigate these areas at a smaller scale (intervals of 20 cm).

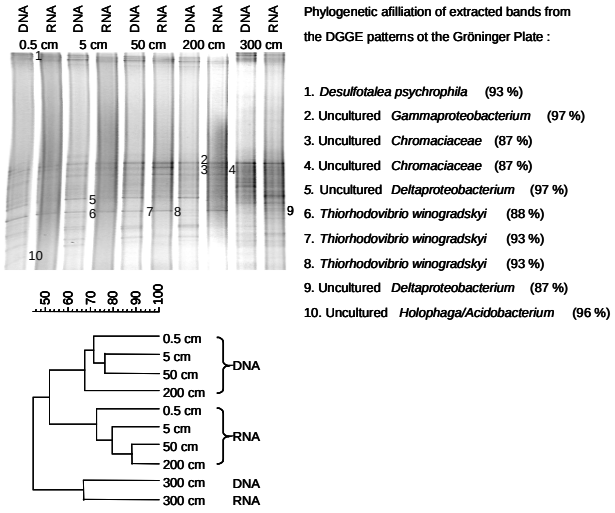

Fig. 2. Comparison of 16S rDNA and 16S rRNA in a core from the Gröninger Plate. The dendrograms were calculated by Pearson correlation and UPGMA.

We will also expand the sequencing of bands to get more information on the microorganisms that disappear in or are unique to the deeper parts of the sediment. More cores will be taken from other sites of the tidal flat to corroborate our initial observations. In future sampling campaigns, we will collect more physico-chemical data to find indications of physiologically specialized microorganisms. We also hope to find out if there is a correlation between physico-chemical properties and the decrease of microbial diversity with depth.

The comparison between 16S rDNA and 16S rRNA derived DGGE patterns seems to be an appropriate method to distinguish active and inactive microorganisms in this habitat. Here, a change in the structure of the DGGE patterns between 2 m and 3 m is also visible. Down to 2 m there are many organism that seem to be abundant but not active. The RNA patterns indicate that at a depth of 3 m the most abundant bacteria, as indicated by the DNA patterns, are also the active ones. We will validate our data with other activity markers, such as phospholipid analysis or enzyme activity measurements.

Acknowledgements

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