DIVERSITY PATTERNS OF AGGREGATE-ASSOCIATED AND FREE-LIVING BACTERIAL COMMUNITIES IN THE GERMAN WADDEN SEA

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Introduction

In the water column of pelagic environments, bacteria occur either free-living or attached to macroaggregates (>500 µm) or microaggregates (<500 µm). The important role of bacteria in the energy flux and nutrient cycling on aggregates in marine and estuarine ecosystems was recently reviewed by SIMON *et al*. (2002). Whereas macroaggregates predominantly appear in open oceans, coastal environments are characterized by high numbers of microaggregates. In the German Wadden Sea, a shallow water column, terrestrial and fresh water input as well as strong tidal currents build a highly dynamic system influencing the aggregate size, abundance and aggregation processes. Despite many investigations of physical and biogeochemical processes in the Wadden Sea, only little is known about which bacterial species are relevant for decomposition of organic matter in the water column.

Marine pelagic microbial communities consist of several taxonomic groups and often show high diversity (DELONG *et al*., 1993; EILERS *et al*., 2000). The *α-Proteobacteria* and *Bacteroidetes* phyla belong to the main phylogenetic groups of aggregate-associated and free-living bacteria in the marine environment (GIOVANNONI & RAPPÉ, 2000). In order to assess relevant species of these groups in the Wadden Sea and their tidal to seasonal dynamics samples were collected and analysed by using specific primer sets for PCR of 16S rRNA genes and subsequent Denaturing Gradient Gel Electrophoresis (DGGE; MUYZER *et al*., 1998). In addition, biochemical and phytoplankton data were used to determine relationships between DGGE banding patterns and alteration of particle and algae composition. Based on the sequence information of excised DGGE bands we were able to identify relevant organisms.

Materials and methods

Samples were taken during diurnal cycles in the backbarrier tidal flat system near Spiekeroog Island on board RV Senckenberg in March, May, June, August and October, 2002 and on different locations in the German Bight in June, 2002. Two fractions were separated by filtration: a) the aggregate-associated bacteria on a 5.0 µm Nuclepore filter, and b) free-living bacteria on 0.2 µm Nuclepore filter. Bacterial cell counts were determined by DAPI staining and epifluorescence microscopy (CRUMP *et al*., 1998). The composition of the aggregate-attached and free-living bacterial community was investigated by DGGE of 16S rRNA gene fragments. Primer sets for specific bacterial groups (*Flavobacteria/Sphingo-bacteria* [JASPERS *et al*., 2001]*, α-Proteobacteria* [this study]) were applied to enhance the detection of relevant species. Cluster analysis of DGGE banding patterns was performed using GelCompare III (Version 2.5, Applied Maths). Curve-based calculation was performed using Pearson correlation and UPGMA. As indicators for quality changes of particulate matter, dry weight (DW) and ratio of particulate organic matter (POM) were determined as described by LUNAU *et al*. (this volume).

Results and discussion

Tidal influences on the bacterial community

Whereas changes of particulate matter in the German Wadden Sea follow tidal dynamics for DW and POM/DW ratio as shown by LUNAU *et al*. (this volume) no changes were detectable for the attached and free-living bacterial community (SIMON *et al*., this volume). Neither DANN- nor RNA-based amplification with eubacterial as well as specific primer sets showed diurnal changes. Thus, we assume that the composition of the microbial community changes occurs on a larger scale and is less affected by diurnal forces.

Seasonal influences on the bacterial community

On a seasonal scale, high variation of particulate matter was detected as shown ba aggregate abundance, DW and POM/DW ratio as well as phytoplankton composition and chlorophyll a values (LUNAU *et al*., this volume; SIMON *et al*., this volume).

Both bacterial abundance and composition also varied during the year 2002 as shown by cell counts and molecular analyses. Free-living bacterial cell counts ranged from 2.8 x 10^6 cells ml^{-1} in spring and autumn to 6.4 x 10^6 cells ml^{-1} in summer concurrent to changes of POM/DW ratio (Figs. 1 and 2). Cell counts of attached bacteria were less variable and ranged between 9.5 x 10^5 cells ml^{-1} in spring during an algal bloom and 6.4 to 4.5 x 10^5 cells ml^{-1} in summer and autumn.

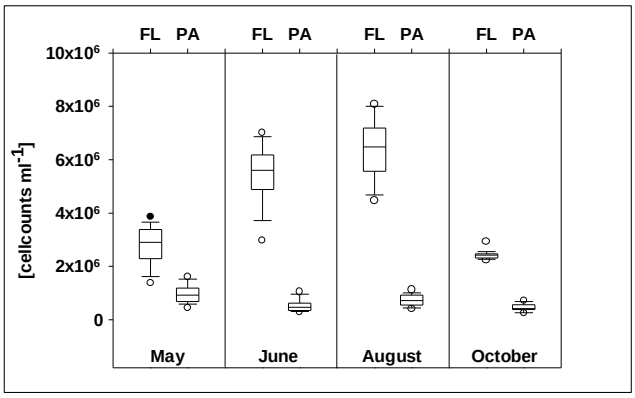

Fig. 1. Cell counts of free-living and aggregate-associated bacteria in various seasons of 2002 (FW=free-living, PA=aggregate-associated).

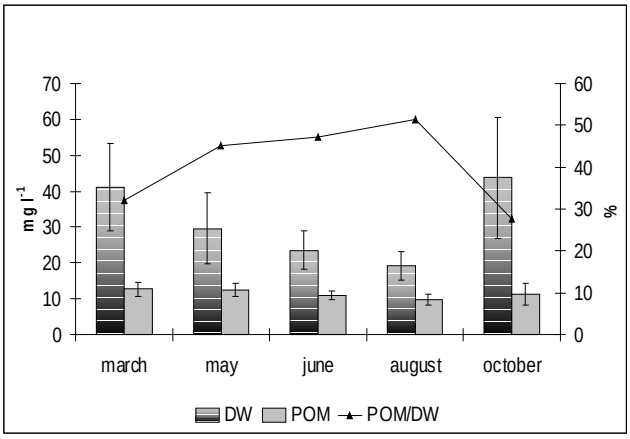

Fig. 2. Dry weight (DW), particulate organic matter (POM) and POM/DW ratio in various seasons of 2002.

The composition of the bacterial community also differed between the two fractions (Fig. 3). Banding patterns of free-living bacteria showed low richness (up to 9 bands per lane) with only few seasonal changes. For the particle-attached bacterial community, banding patterns showed higher richness (up to 14 bands per lane) with larger variations during the year.

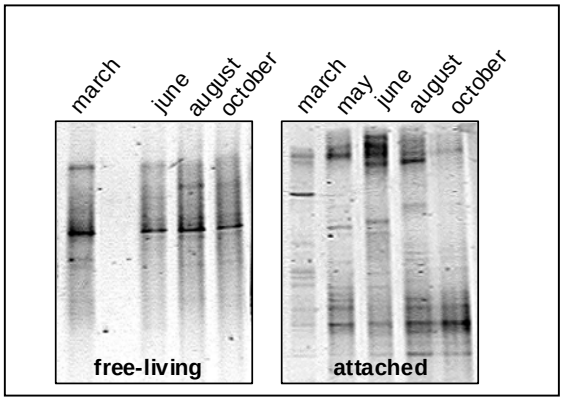

Fig. 3. DGGE banding patterns of eubacterial 16S rDNA fragments of free-living and aggregate-associated bacterial communities in various seasons of 2002.

A comparison of samples from the years 1999, 2000 and 2002 showed covariations in the DGGE banding patterns (validated by sequencing of excised bands) indicating that some organisms are able to persist independently of seasonal, biological or physical changes of the environment.

DGGE banding patterns of *α-Proteobacteria* showed two bands which were almost permanently present in all seasons from 1999 to 2002 (Fig. 4).

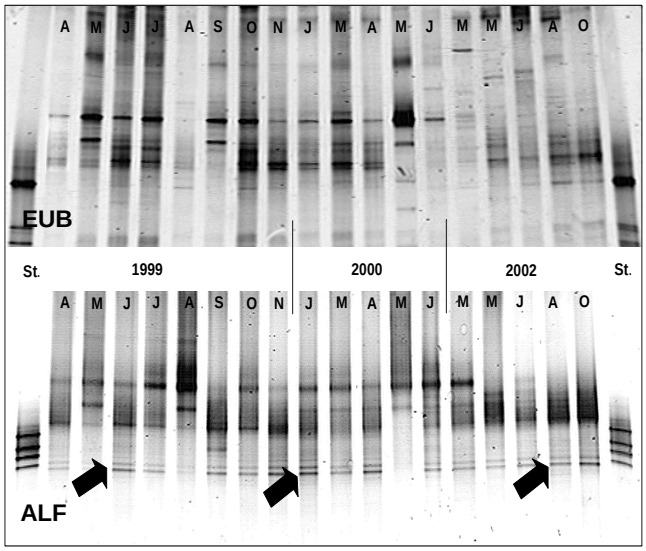

Fig. 4. Comparison of aggregate-associated bacterial communities for three years in the German Wadden Sea. Arrows show two bands permanently present in samples of all years. (EUB=eubacterial primer set, ALF=primer set specific for *α-Proteobacteria*).

In contrast, some bacterial species appear solely at particular times. During an algal bloom in the year 2000 bacterial community changed successively in parallel to the composition of diatoms (Figs. 5 and 6a). Application of a group-specific primer set for *Flavobacteria/Sphingomonas* showed a high richness in DGGE banding patterns of the attached bacteria (Fig. 6b). With increasing abundance of *Guinardia delicatula*, the community composition of bacteria belonging to *Flavobacteria/Sphingomonas* changed significantly. This group is known as consumers of polymeric compounds (KIRCHMAN, 2001) and is therefore important for particle decomposition. Thus, changes in organic matter are directly reflected by the bacterial community.

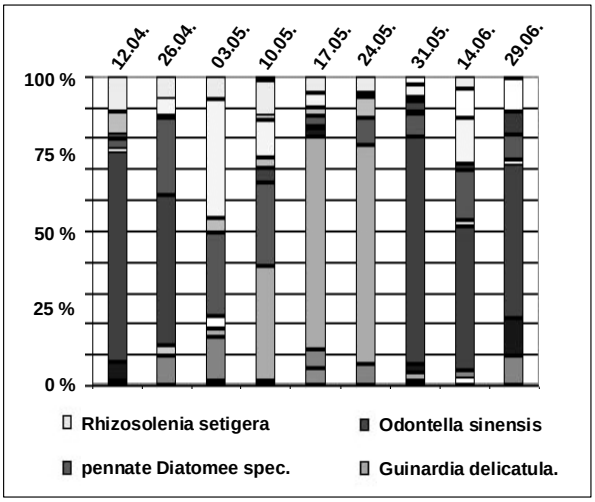

Fig. 5. Changes of algae composition during an algal bloom in the German Wadden Sea (April – June 2000).

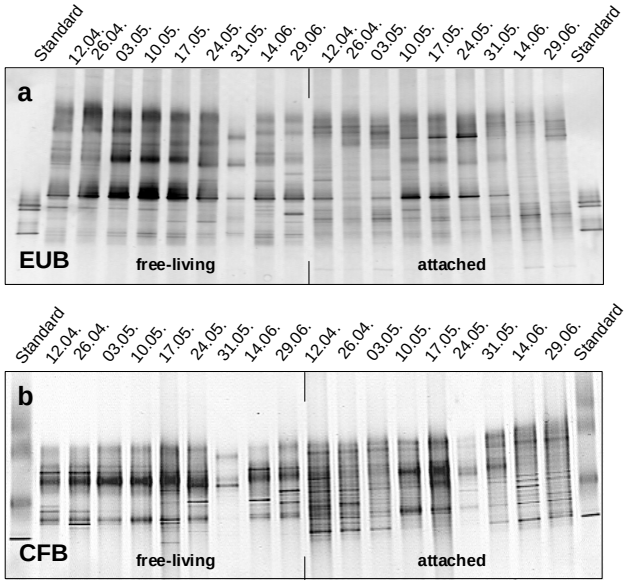

Fig. 6. Alteration of aggregate-associated and free-living bacterial communities during an algal bloom (April – June 2000) (EUB=eubacterial primer set, CFB=primer set specific for *Flavobacteria/Sphingomonas*).

Comparison of German Bight and Wadden Sea communities

To determine differences between bacterial communities of the open North Sea and the Wadden Sea samples taken at different stations (see Fig. 7) were analysed by DGGE and cluster analysis. The results of the analysis show distinct clusters for free-living and particle-attached bacteria irrespective of the sampling station (Fig. 8). Furthermore, samples taken at different times at the same location always show high similarity as found earlier for diurnal cycles (see above).

Wadden Sea samples were most similar to North Sea samples taken at station GB 5 close to Spiekeroog Island for free-living as well as for particle - associated communities.

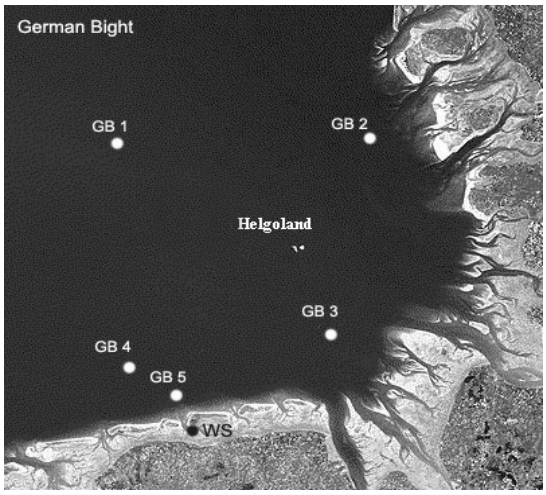

Fig. 7. Spatial sampling in the German Bight in June, 2002. (GB 1 – 5: German Bight sampling stations, WS=Wadden Sea sampling station).

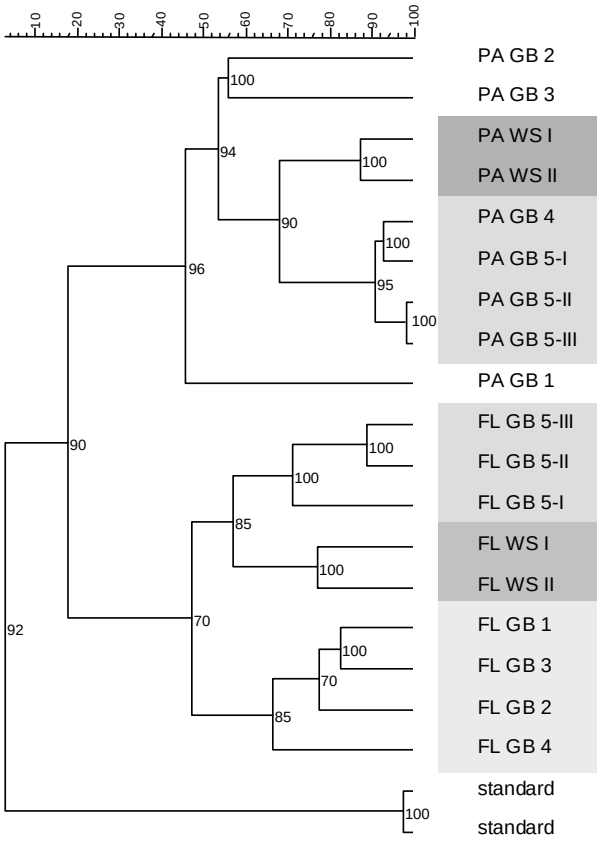

Fig. 8. Analysis of DGGE results of spatial sampling in the German Bight and Wadden Sea using cluster analysis (PA=Particle-Associated, FL=Free-Living, GB=German Bight sampling station, WS=Wadden Sea sampling station, I-III=different sampling times during a tidal cycle at the same station).

These results indicate an exchange between the German Bight and the backbarrier tidal flat system. In contrast, bacterial communities of stations further north in the German Bight were distinctly different, indicating less exchange processes between the coastal area and the open North Sea.

Conclusions

The results of the studies in the backbarrier tidal flat system of Spiekeroog Island show differences between free-living and attached bacterial communities. No changes were observed for the various communities within tidal cycles, whereas seasonal variations were obvious, depending on biological, biochemical and physical changes of the environment. DGGE analysis of specific bacterial groups allows a more detailed insight in the bacterial community and shows high richness of species. A high similarity of Wadden Sea bacterial communities and bacteria of the German Bight was observed in the coastal area near Spiekeroog Island. Within the German Bight, the bacterial communities on the particles and the communities in the free water were more similar among each other than the communities of both fractions at one sampling station.

Outlook

Our results lead to further questions about the ecological function of the abundant species. Therefore, we will combine sequence information from DGGE banding patterns with sequences of isolated strains from the Wadden Sea obtained from another study (STEVENS *et al*., unpublished results). Specific oligonucleotides for 16S rRNA genes will be designed for the use in fluorescence *in-situ* hybridization and real-time PCR to determine the abundance of selected species. Based on the results of biochemical investigations of particulate matter, molecular methods will be combined with microautoradiography and hydrolytic enzyme measurements to study particular degradation processes in the Wadden Sea.

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