Proposal to apply the 454 technology to coastal marine sediments

Submitted by: Alban Ramette, Angelique Gobet and Antje Boetius, MPI Marine Microbiology, Bremen, Germany (aramette@mpi-bremen.de)

Abstract

The microbial ecology of coastal zones of the oceans is still poorly understood, despite the fact that sandy sediments represent a major proportion of coastal zones, and that microbes play a key role in the global cycles of carbon and nitrogen. We propose to apply the 454 massive tag sequencing to a set of well-characterized sandy sediment samples of Northern Germany for which we already have extensive data concerning the diversity of the bacterial communities and contextual environmental parameters measured through space (i.e., horizontal and vertical spatial samplings) and time (eight seasons). Combining the 454 technology with the existing data will enable a better understanding of the extent of diversity in those poorly understood, yet important ecosystems. The identification of significant environmental factors which may be associated with changes in the rare biosphere may generate new hypotheses about the ecology of those microbes. Comparison of the massive tag sequencing technique with other community fingerprinting techniques will enable a further validation of the new strategy.

Introduction

In recent years, an increasing number of microbial studies have addressed the role of space and time in generating diversity patterns in microorganisms. However, the relative contribution of spatio-temporal effects vs. variation of physical and chemical parameters on diversity is still poorly understood, especially in marine sediments.

The coastal zones of the ocean are highly productive ecosystems, which despite their relatively small area, play a large role on the global cycles of carbon and nitrogen. Biodiversity and species composition are changing at an increasing rate due to coastal development, eutrophication, pollution, exploitation, species introduction and possibly global warming. Hence, a thorough understanding of the transport processes, material flows and biodiversity within these ecosystems is needed.

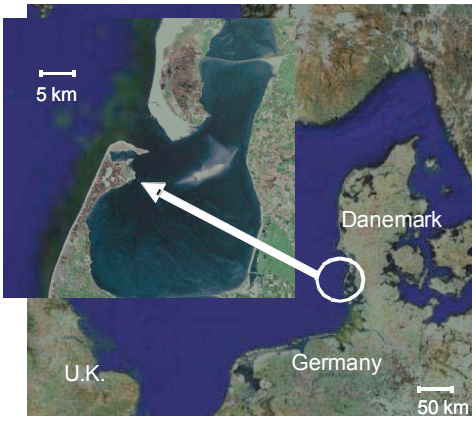

Fig. 1. The Sylt study site (54°60′N 8°26′E)

The proposed project will focus on coastal areas of the North Sea island Sylt (**Fig. 1**), where long-term studies are available. Special features of this coastal ecosystem are the substantial temperature changes throughout the year, the impact of storms, the strong pelagic phytoplankton bloom in spring, and the benthic diatom bloom in spring and autumn.

A well-characterized set of samples

Field site description. Sediment cores were collected at a subtidal sand flat in the Sylt-Rømø Basin (North Frisian Wadden Sea, Germany; **Fig. 1**) over eight seasons up to 15-cm depth (one-cm layer resolution). A spatial sampling design was also followed and included a 150×80 m-long transect with ten stations that were sampled at three depths each in a collaborative project between the Microbial Habitat group (MPI) and the AWI biogeochemistry lab (van Beusekom) at Sylt.

Available contextual information. High-resolution biogeochemical analyses were performed to characterize the samples (**Fig. 2**). The set of parameters already available encompasses total cell number estimations (acridine orange counts), extracellular enzymatic acitivities (α-glucosidase, β-glucosidase, chitobiase, lipase, phosphatase, aminopeptidase), growth rate estimations (^{3}H-thymidine, ^{14}C-leucine, ^{14}C-Synechococcus), benthic photopigments (chlorophyll a and phaeophytin), extracellular polysaccharide (EPS) concentrations, sediment permeability and C/N ratios. Pore water samples were also taken to determine salinity and nutrient concentrations ($Si(OH)_4$, PO_4^{3-}, NH_4^+, NO_3^- and NO_2^-), and water column data were obtained from the Sylt time series (Justus van Beusekom, Alfred Wegener Institute).

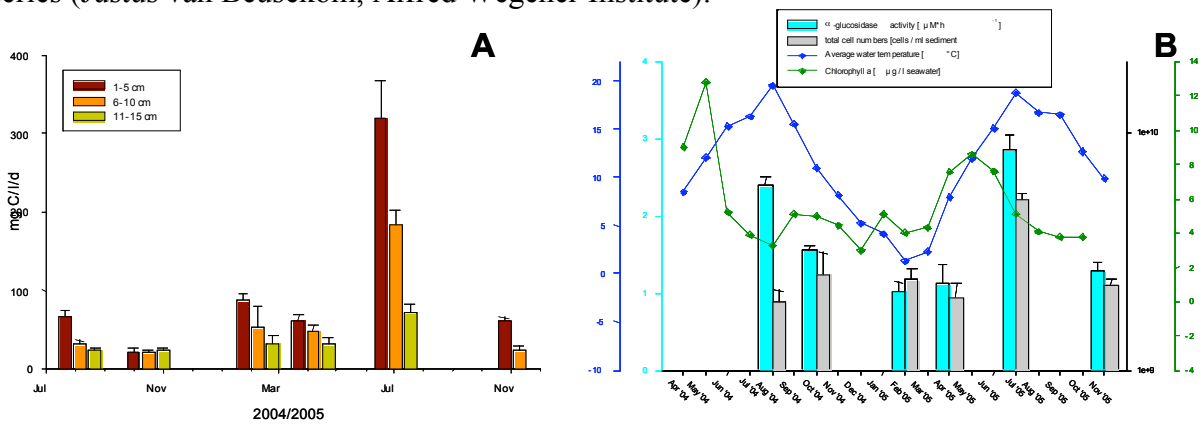

Fig. 2. Examples of depth and season-related fluctuations at the study site. **A)** Seasonal changes in bacterial carbon production based on thymidine incorporation. **B)** Seasonal variation of total cell numbers, α-glucosidase activity, chlorophyll a and average water temperature (Boer, Ramette et al., unpublished data)

Molecular characterization of the samples. DNA was extracted and purified from all samples and is directly available for further molecular investigations. Changes in bacterial community structure were determined using the Automated rRNA Intergenic Spacer Analysis (ARISA) method, which offered a high-resolution description of the variation in community diversity (**Fig. 3**).

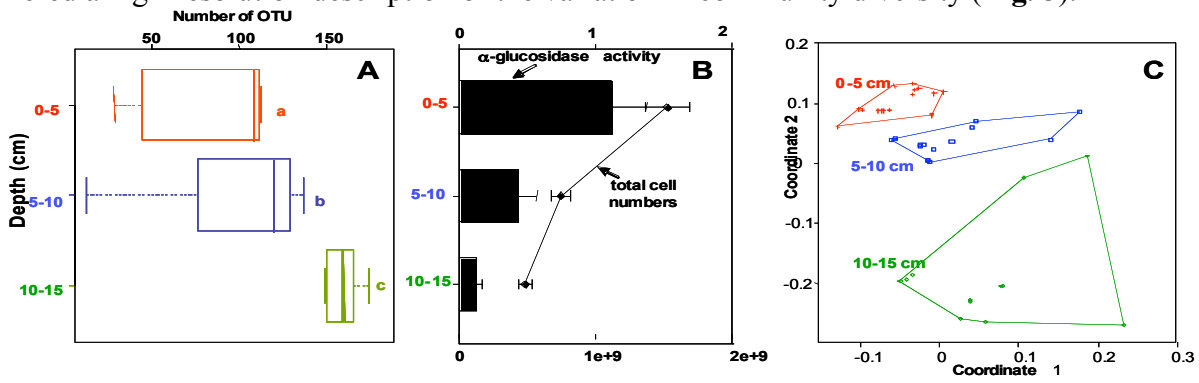

Fig. 3. ARISA fingerprinting results. **A)** Number of Operational Taxonomic Units (OTU) obtained at different sediment depths. **B)** measurement of associated total cell numbers and enzymatic activity (only one is depicted here). **C)** Non-metric multidimensional scaling (NMDS) plot of the similarity between samples based on ARISA fingerprints (Boer, Ramette et al., unpublished data)

Molecular fingerprinting revealed the presence of an increased diversity with sediment depth, which was associated with a decrease of cell numbers (Fig. 2B, 2C). This observation should be further confirmed by using a more in-depth fingerprinting strategy such as the 454 technology to identify the extent of the rare biosphere.

In addition, we are currently sequencing 16S rRNA genes and the associated ITS regions to better link ARISA data with a taxonomic description of the samples. This would also prove valuable when shorter tag sequences are used to analyze samples, and this would enable a direct identification of the sequences retrieved in common by the different approaches.

Multivariate analyses. Multivariate analyses are needed to identify the relationships between biotic and abiotic variables, and to determine either the individual OTU (**Fig. 4A**) or the specific samples (**Fig. 4B**) which are associated to a set of environmental parameters. It is then possible to assess how such taxa-environment interactions are stable spatially and temporally.

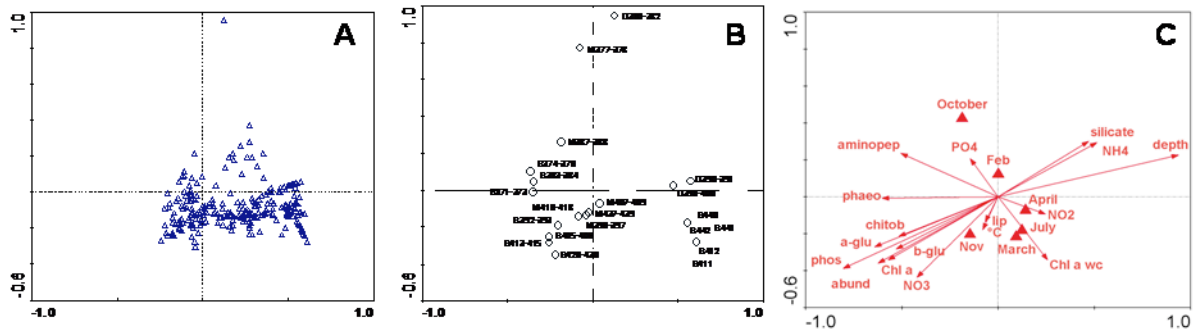

Fig. 4. Canonical correspondence analysis by OTU (**A**), samples(**B**) and environmental parameters(**C**). Arrows indicate the direction of the gradients for the environmental variables. The longer the arrow, the higher its impact on biological variation. (Boer, Ramette et al., unpublished data)

Such numerical techniques enable the extraction of the meaningful information out of complex datasets and the testing of new hypotheses regarding causal relationships between community diversity and environmental factors. The relationships are then tested for significance by Monte Carlo permutations which enable a more robust and distribution-free evaluation of the results.

Conclusions. The bacterial community composition in Sylt sandy sediments was found to be highly variable. However, no single factors alone influenced community structure, but a complex interaction of different environmental parameters was evidenced as affecting community composition. In order to further determine the nature and the relative importance of the factors that affect microbial communities in sandy coastal ecosystems, our experimental setup and high throughput sequencing technology will certainly represent an excellent combination.

Proposed experimental plan

In order to better understand the environmental drivers of the diversity of microbial communities in sandy coastal ecosystems and especially those affecting the rare biosphere, we propose the following:

1) To apply the 454 massive tag sequencing technology to the well-characterized Sylt samples to identify the extent of microbial diversity.

2) To identify and quantify the effects of the major environmental factors by using the extensive contextual data available. This will enable a better understanding of the relationship between diversity patterns, space-time scales, and environmental parameters, especially when rare biotypes are included in the description of complex ecosystems. Interesting lines of investigation could include the assessment of whether rare biotypes specifically respond to certain environmental factors, spatial scales, and if such determinism is reproducible through time. This will thus shed light on their putative ecology.

3) To compare diversity patterns obtained by the 454 technology and traditional fingerprinting methods such as ARISA, terminal-Restriction Fragment Length Polymorphism (T-RFLP), and traditional ribosomal sequencing approaches. It will then be possible to estimate whether our description of microbial diversity and ecology fundamentally changes when a more in-depth description of diversity is used.

4) To use in-house bioinformatic facilities (supported by the group of Frank Oliver Glöckner @ MPI) to deal with the massive amount of short sequence data generated (e.g., storage, cleaning, evaluation, database creation) and create standard analysis pipelines.

Sample preparation, Time-schedule, personnel

We expect to perform the massive sequencing relatively quickly, since all community DNA is available and suitable for molecular work. The DNA extraction procedure was done according to the MOBIO DNA extraction protocol (MO BIO Laboratories). One g of sediments was generally used for each sample and the final extracted and purified genomic DNA was stored at -20C in 50 µl of Tris-EDTA buffer. All DNA samples were of good quality (A260/A280>1.6) for subsequent molecular applications. We can send 10 µl subsamples (ca. 15 ng DNA/µl).

The total number of samples covering the complete spatial and temporal sets is in the order of 70, but we can select a subset of 16 for a first analysis. We currently have a finishing PhD student (Simone Boer) who has worked on the initial environmental and molecular characterization of the Sylt samples, and a new PhD student (Angelique Gobet) who just started few months ago. Angelique will work further on the sequencing and molecular characterization of the Sylt samples and would directly work on the 454 data if available. Alban Ramette supervises the multivariate analyses and dataset comparisons, and helps build the data pipelines in collaboration with Frank Oliver Glöckner's group. Antje Boetius leads the project on dynamics in microbial diversity and function in coastal sands of Sylt.