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Overview

The overall aim of WP 1 is to identify the linkages between different environmental conditions and the genetic blueprint (which includes the taxonomic profile as well as the functional potential and the realized, specific activities) of microbes in the Baltic Sea along temporal and spatial gradients.

This deliverable reports two finished studies that were performed in the framework of the BONUS BLUEPRINT project in order to target spatiotemporal variability of the genetic blueprint relative to environmental conditions in the Baltic Sea. Besides different types of anthropogenic impacts, the abiotic gradients of salinity and oxygen have a major influence on the microbial communities and their activities. Both studies focus on the detection and analyses of communities that establish along the salinity gradient in the Baltic Sea. For analysing the impact of anthropogenically mediated factors it is a precondition to have a proper understanding of the variability in microbial communities associated with the natural environmental gradients in the Baltic Sea as well as of their responses to disturbances in these factors.

Study 1 (Bennke et al.) describes the development of a monitoring system for phytoplankton, using chloroplast sequences which are obtained by general 16S rRNA gene-based diversity analyses. This was successfully tested along the spatial salinity gradient of the Baltic Sea.

Study 2 (Shen et al.) used an ANOVA (analyses of variance) to identify bacterial taxa that, according to the abundance of their 16s rRNA gene sequences, were able to adapt to different salinities after short-term exposure (4 days) to salinity changes. This statistically supported linkage of the genetic blueprint with different environmental conditions, as well as the identification of specialist taxa which showed a consistent response to a particular salinity, may be used in future studies as potential indicator

taxa. These can potentially constitute another important indicator parameter besides the identified indicator genes (D4.3). We have furthermore statistically explored mechanisms that determine the assembly of the detected specialist taxa as well as generalist taxa (which show no salinity preference) by analyzing phylogenetic relatedness of their 16s rRNA gene sequences. Using the 'genetic blueprint' of bacterioplankton to understand assembly mechanisms is important to better predict compositional changes in response to a changing environment and to specific disturbance scenarios. Study 2 includes both spatial and temporal aspects by investigating the genetic blueprint of bacterioplankton from different Baltic Sea sites after environmental change.

Study 1:

Bennke, C. M., Pollehne, F., Müller, A., Hansen, R, Kreikemeyer, B. & Labrenz, M.
The use of a general 16S rRNA primer system to identify phytoplankton throughout the Baltic Sea

Abstract:

Due to the evolutionary relationship between *Cyanobacteria* and chloroplasts of all oxygenic photoautotrophs, both are amplified with prokaryotic 16S rRNA gene primers. Chloroplast sequences can make up as much as 50% of a 16S rRNA library, but because of the comparably low phylogenetic resolution within limited chloroplast databases they are usually removed from further analyses. However, chloroplast 16S rRNA databases are constantly improving and our aim was to proof if meanwhile whether the combined 16S rRNA gene and 16S rRNA sequences of phototrophic prokaryotes and eukaryotes generated by a general bacterial primer set could be used to characterize their distribution *in situ*. Based on the phytoREF database this was performed for samples throughout the Baltic Sea, from which phytoplankton > 20 µm was also identified by microscopy, allowing a comparison and evaluation of these different approaches. In general, both methods revealed similar distributions of diatoms, chlorophytes, and filamentous cyanobacteria. Due to for instance kleptoplastidy, the molecular analysis of dinoflagellates suffered from uncertain plastid allocation by some species, whereas light microscopy is inherently limited in the identification of smaller cells. However, 16S rRNA and microscopic analyses together provided a comprehensive overview of the phototrophic community, demonstrating the usefulness of this combined method as a tool in monitoring/assessment strategies.

Study 2:

Shen, D, Jürgens, K & Beier, S. Changes in salinity affect the community assembly of bacteria with different life strategies.

Abstract:

The response of local communities to changes in salinity and the processes that underlie community assembly are unclear, particularly with respect to shifts in the phylogenetic patterns of bacteria differing in their life strategies. Marine-freshwater transitions are environments well-suited to the study of microbial assembly mechanisms, as there is little overlap in the abundant bacterial taxa present in each of these ecosystems. Here, we implemented a transplant

experiment in which bacteria originating from freshwater, brackish and marine regions of the Baltic Sea areas were reciprocally incubated under the other two conditions and thus exposed to a change in salinity. Our results showed that after these incubations the bacterial assemblages contained a greater abundance of specialist taxa than the generalists. Originally common taxa were overrepresented among taxa that were specialists in their native environment. In some cases, taxa specialized to a non-native environment were largely recruited from originally common taxa. Initial taxa abundance did not seem to be relevant to the growth behavior of generalists. Most of the specialists were more closely related than expected by chance, suggesting the importance of habitat filtering for their assembly. Generalists, by contrast, were distantly related, indicative of facilitative interactions.

The two manuscripts, which are both currently under review in peer-reviewed scientific journals, are deposited on the internal page of the BONUS-Blueprint homepage (http://blueprint-project.org/). They will be accessible to BONUS personnel, by using an internal password. Upon acceptance the manuscripts will be inserted below and thereby be publicly available.