D1.1. Insights into linkages between blueprint genomic information and environmental status in selected samples (with **WP4** and **WP5**) (**Month 18**)

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Introduction

This deliverable summarizes some first results from the activities in work package 1 (WP1) giving insights into linkages between blueprint genetic information and environmental status in selected samples.

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. Besides different types of anthropogenic impacts, the abiotic gradients of salinity and oxygen have a major influence on the microbial communities and their activities. In the presented study our focus was on selected samples along the horizontal salinity gradient in the Baltic and the 'behavior' of intrinsic bacteria along this environmental gradient. Similar studies on the response towards changes in oxygen concentration are currently being processed.

Salinity has been identified as a major factor controlling bacterial community composition as well as the distribution of different phylotypes based on investigation of their ribosomal and functional genes (Logares et al., 2009; Beier et al., 2011; Herlemann et al., 2011). However, little is known if and in how far salinity affects community functioning, i.e. if a certain level of salinity is coupled to a specific signature in the functional blueprint of prokaryotic communities.

Changing salinity is a relevant environmental scenario in the Baltic Sea, where i.e. salt inflow events from the North Sea periodically increase salinity. On the other hand, increased river run off due to anthropogenically caused changes in climate and precipitation are predicted to decrease salinity.

It is essential to investigate the impact of salinity on microbial genomics alone in order to untangle its effect from the impact of other factors that influence the Baltic Sea environmental status, and which sometimes covary with salinity such as oxygen depletion, pollution and other stressors.

To investigate the influence of changing salinity on bacterial communities and their functioning, we have setup a full-factorial transplant experiment where bacterioplankton communities from freshwater, brackish, and marine sites of the Baltic Sea were incubated under each other's environment.

We aim to address two general questions: (i) are shifts in the bacterial community induced by changes in salinity correlated with functional changes and (ii) how do locally salinity-adapted bacteria respond to new environments?

Experimental Setup

Water samples for the transplant mesocosms were taken during the cruise AL439 (RV Alkor) from 06/04/2014-06/19/2014 from the Skagerrak (marine conditions), the central Baltic (brackish conditions) and the Gulf of Bothnia (freshwater conditions) (Figure 1). This cruise was performed in collaboration with scientist from WP6, who will analyze the microbial genomics of in situ samples to deduce further linkages to different environmental situations in the Baltic Sea. Water from the sample sites was directly filtered for later nucleic acids extractions and stored in liquid Nitrogen. Water for the experiment media was 0.2 µm filtered and stored in dark while the used filters were frozen at -20°C. Water for the experiment inocula was filtered 0.8 µm pore size and stored at 4°C during until the experiment started back in the IOW laboratory.

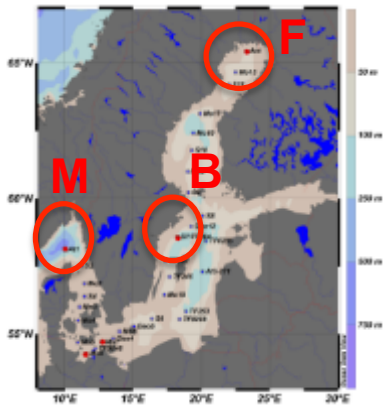

Figure 1: Map of the Baltic Sea with the sample sites for marine (M), brackish (B) and freshwater (F) conditions.

The transplant experiment was started on 07/01/2014. For both, the media and inocula the above-mentioned filtration steps were repeated one day before the experiment started. In order to minimize effects of different nutrient conditions in the different media, nutrients were added to the inocula. These nutrients were derived by autoclaving the stored 0.2 um filters collected after the first filration (on-board). For this purpose all filters were placed together in a volume of about 200 ml MilliQ water. After autoclaving the MilliQ water - now containing debris of the filters and filtered material - was filtered (0.2 µm pore size) and the resulting suspension was distributed into three equal volumes, which were then added to each inoculum.

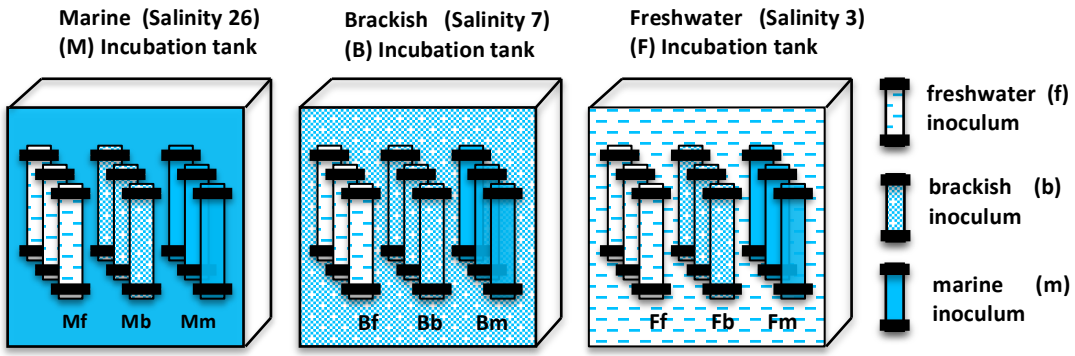

Figure 2: Experimental design of the transplant mesocosms. (Capital letters indicate the respective incubating medium and small letters indicate the respective inoculum.)

In a full factorial design with triplicate treatments 3L of each inoculum were placed within dialyses bags into 100L of each medium (Figure 2). Water for later nucleic acid extractions was filtered (0.2 µm pore size) directly from the inocula and from each treatment after 4 days of incubation. Salinity, pH, nutrients, cell counts and bacterial production (thymidine incorporation rates) were measured daily. We further have used BIOLOG's microplates that were inoculated each day of the experiment to assess the carbon utilization potential which was used as a proxy for community functioning.

DNA and RNA were extracted and have been sent for 16S rRNA gene amplicon sequencing (DNA, each triplicate), Metagenomics (DNA, for each treatment with pooled triplicates) and Metatranscriptomics (RNA, for each triplicate). We have retrieved and analyzed all sequencing data from the amplicon sequencing approach. However, because of technical problems at the sequencing facility we so far have received only a subset of metagenome sequences and no metatranscriptome sequences.

Preliminary Results

Correlation of taxonomic and functional data

For testing to what extend the taxonomic data were correlated to the community functioning detected by the BIOLOG's approach, a Mantel test (Mantel, 1967) was employed.

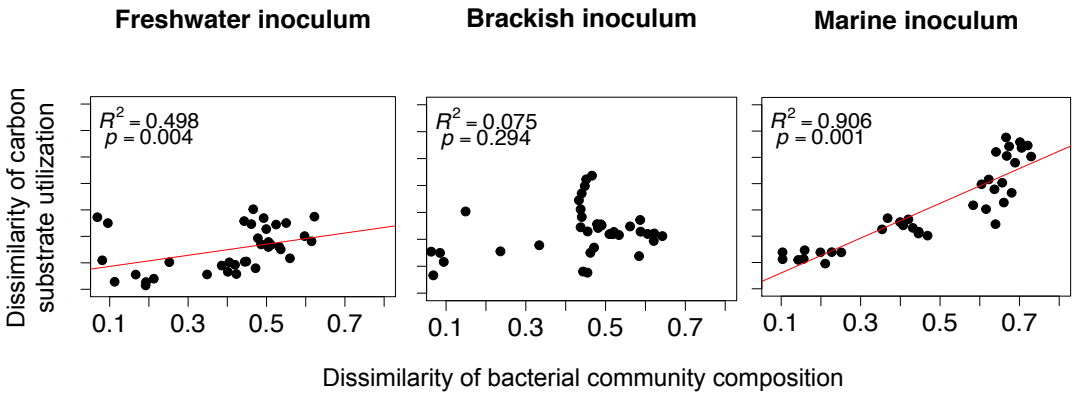

Figure 3: Mantel tests based on Bray-Curtis dissimilarity matrices indicating the strength of correlation between the functional and the taxonomic profile in samples that were derived from the same inoculum. Operational taxonomic units for the taxonomic profile have been determined by a 97% similarity cutoff of the sequenced 16S rRNA gene fragment. The functional data presented here were obtained at 96 h incubation of BIOLOG microplates.

Mantel tests revealed significant correlation between functioning and bacterial community composition in the treatments that were inoculated with freshwater or marine inocula, but not those that were incubated with brackish inocula (Figure 3). The missing correlation in the treatments inoculated with brackish bacteria could be due increased plasticity of the community members if functional shifts are stronger then simultaneous shifts in the community composition. Vice versa, if overall shifts in the community composition were higher than simultaneous shifts in the functional profile, this would indicate increased functional redundancy in the brackish community. Both,

plasticity of individual taxa as well as functional redundancy can be estimated quantitatively from metatranscriptome data (Beier et al., 2015). Therefore, future analyses based on metatranscriptome data may offer more detailed information on the mechanisms that cause the observed correlation patterns.

Grouping of taxonomic and functional data

In order to examine if rather the inoculum or the environment was driving the final community composition as well as the functional profile in our treatments the similarity of 16S rRNA gene amplicon data and data derived from the BIOLOG's plates was visualized in a NMDS biplot (Figure 4).

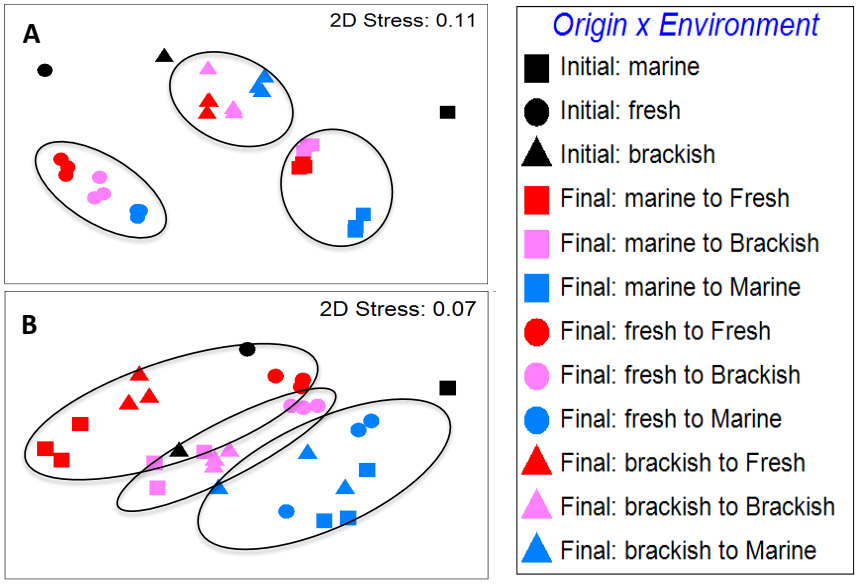

Figure 4: NMDS biplots based on Bray-Curtis dissimilarities of the taxonomic (A) and functional (B) profile from the initial inocula and the treatments after 4 days of incubation. Operational taxonomic units for the taxonomic profile have been determined by a 97% similarity cutoff of the sequenced 16S rRNA gene fragment. The functional data presented here were obtained at 96 h incubation of the BIOLOG microplates that were photometrically screened for cell growth.

Salinity has been reported as a major driver for bacterial community composition. Still, our data show that after 4 days incubation of different inocula in different salinity regimes, in all cases the inoculum was more important for the final community composition then the incubation environments (Figure 4A), which were characterized by different salinities. On the other hand, the functional profile – here derived from utilization of different carbon substrates – was more similar from samples if they were derived from the same environment, independent from the inoculum (Figure 4B).

In contrast to the data obtained from BIOLOG plates, metatranscriptomic analyses will provide a more comprehensive view on the functional response, including the whole community metabolism, and, more important, are not based (and biased) by cultivation. Still, also our current results, with functional profiles based on BIOLOG derived data, indicate that at a short-term timescale (few days) functional data may reflect the environmental conditions after disturbances with higher sensitivity then taxonomic data.

Ecological strategies of taxa

One of the overall goals of the BLUEPRINT project is to identify key genes that are linked to certain ecosystem functions or taxa, which sensitively indicate a change of the environmental conditions. We therefore have used the abundance dynamics of bacterial taxa of the same source-inocula to examine the response of taxa to new salinity-induced environments. The abundance of taxa that were defined by OTUs (clustered at 97% sequence similarity) was estimated by multiplying the relative abundance of each OTU with the number of cells per volume water (Andersson et al., 2010).

ANOVA analyses indicated that about 50 % of the tested taxa in each inoculum were sensitive to salinity changes by significantly changing their abundance. About half of these sensitive taxa of each inoculum were characterized by highest abundance in the native environments (Figure 5). However the other half of the sensitive taxa featured highest abundance in some other but not the native environment (Figure 5).

The observation of a high fraction of bacterial taxa with highest growth rates at a salinity different from the origin of the inoculum indicates the importance of a seed-bank for the community dynamics after a disturbance (Figure 5).

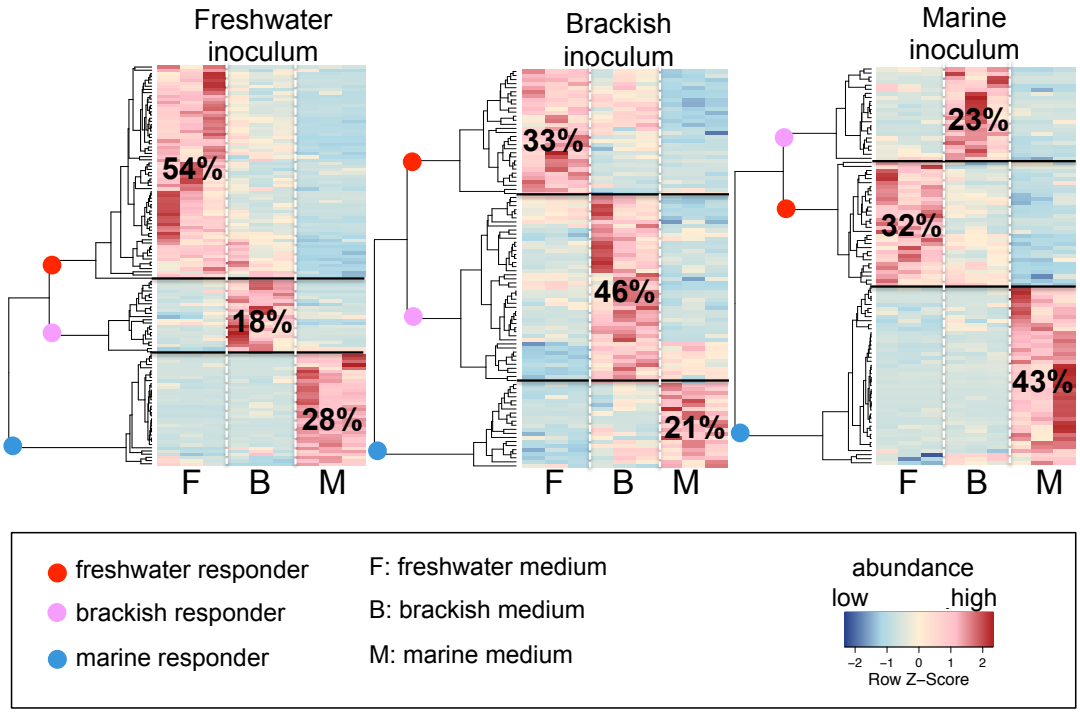

Figure 5: Heatmaps and dendrograms showing the abundance of sensitive taxa from each inoculum along the different salinity treatments

Outlook

Due to the current lack of a complete set of metagenomic and metatranscriptomic data, we did not yet start the anticipated collaboration with WP4 and WP5. Still, our current preliminary results give some insights into linkages of the blueprint of genomic information based (based on 16S rRNA genes) and environmental status.

Future metatranscriptome data of the samples will be used to construct a functional profile similar to that derived from the BIOLOG's data but based on abundances of transcripts for genes covering the total functional response of the community. The

analyses based on BIOLOG's data will be repeated with the transcript functional profile and refined by grouping transcripts into functional categories as i.e. genes involved into carbohydrate metabolism as defined by the KEGG database (Kanehisa et al., 2007). Metatranscriptomic data will further be used to estimate the influence of functional redundancy and plasticity on the community functional response.

Metagenomic data will be used to screen for common genes in taxa assigned to each coherent ecological group. This approach will help us to answer if taxa comprise genetic capacity to cope with salinity-induced environments.

In order to analyse the impact of reduced oxygen concentrations, which occur nearly permanently in the deeper basins and seasonally also in various coastal areas of the Baltic Sea, we have further conducted another ship-board experiment. There we have simulated the mixing of suboxic and anoxic waters within the redox gradient in the central Baltic Sea in order to study the response of microbial communities and the temporal dynamics of transcriptional patterns induced by changing oxygen concentrations.

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