FROM PURE CULTURES TO BACTERIAL COMMUNITIES: KNOWLEDGE GENERATION BY EXPRESSION PROFILING

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Abstract
The majority of prokaryotes live in marine habitats and play a key role in global element cycling. Yet, most of them are still unexplored and little is known about their functions as well as their reactions to the rapidly changing environment. Plus, they are expected to be a treasure trove for new genes interesting for biotechnology and medical applications. Transcriptomics and Metatranscriptomics are currently the techniques of choice to assess gene expression and to unravel the reactions of the microbes to environmental changes. In this study we present gene expression studies for the marine model bacterium *Rhodopirellula baltica* SH1^T and metatranscriptome studies employing 'Next Generation Sequencing' to analyze seasonal changes in the North Sea.
Keywords: Bacteria, Genetics, Time Series, Monitoring, Surface Waters

The complete genome sequencing of the marine planctomycete *Rhodopirellula baltica* SH1^T revealed many fascinating and rare features like a high number of sulphatases and a global mechanism of gene regulation. In order to gain further knowledge about this intriguing organism, it was cultured under various conditions in chemostats and the resulting gene expression was analyzed with whole genome microarrays. The presented data (Fig. 1) illustrates the reaction of the organism to heat, cold and salinity shocks. Separately for each condition, the gene regulation was monitored during time series, with sampling after 10, 20, 40, 60 and 300 min. The response to heat shock was most pronounced after 40 min, while adaptation to salinity up-shift took 300 min. The strongest overall response was observed in the heat shock experiment. During cold shock, the cells seem to reduce their activity. Hence, the number of upregulated genes is significantly smaller than the number of downregulated ones.

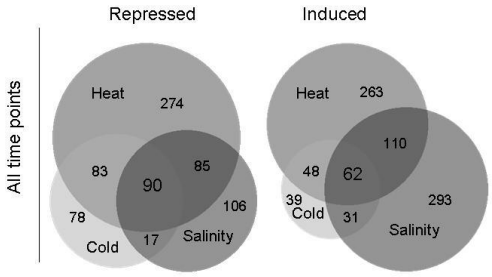

Fig. 1. Stress answer of R. baltica with the number of regulated genes

R. baltica shows a general stress response as well as a specific answer to different stress situations. While heat shock mainly influenced the classical heat shock genes, cold shock also had an impact on genes from the lipid metabolism. In addition, genes involved in recombination, in secondary metabolites biosynthesis, transport and catabolism were induced at enhanced temperature. Interesting was also the upregulation of SecA, which belongs to the extracytoplasmic stress response. In the cold shock experiment, genes for amino acid biosynthesis as well as for protein fate and synthesis were down-regulated, emphasizing the switch to a passive state of the cells. Genes involved in sporulation (oppB) and pilus assembly were repressed, leading to reduced motility and budding ability. The salinity shift changed the expression of genes, which encode for enzymes with a function in ion transport and in morphology. Glutamate and trehalose act as cytoplasmic osmoprotectants, which is why the respective genes involved in the synthesis of these components were upregulated due to the osmotic shock.
The experiment also enabled us to predict new functions to a set of genes without an assigned function by analyzing coregulations with known genes.This will not only enrich the functional information in the databases but might also lead to the discovery of new enzymes for biotechnology or medical applications.

Open questions : Can knowledge gained by pure culture experiments be transferred to natural habitats? Do microorganisms behave similar in the environment and in the lab and how do the over 90% of uncultivable species react to environmental changes?

To answer these questions, the MIMAS "**M**icrobial **I**nteractions in **MA**rine **S**ystems" project was set up. It aims at investigating the seasonal changes in the microbial communities at two long term ecological research sites in the North Sea (Helgoland Roads) and the Baltic Sea (Gotland Deep). The Gotland Deep is known for its stable waterbody and an Oxic-Anoxic intersection at a depth of 120 m. In contrast, Helgoland Roads captivates with its fast exchanging waterbody. In order to explore the biodiversity of the Helgoland Roads research site, single cell *in situ* hybridization and ribosomal RNA sequencing are performed. However, the core of the project is based on a "Meta-Omics" approach: Metatranscriptomics and Metaproteomics will shed light into the active fraction of genes, while Metagenomics will address the genetic potential of the bacterial community as a whole. This integrated approach will give new insights in the ecological role of marine bacterial communities and their response to environmental changes such as climate change.
Metatranscriptome analysis (Fig. 2) will be performed via pyrosequencing to get a comprehensive insight into the flexible gene expression adaptation of the bacterial community due to seasonal changes of their environment. For this purpose seawater is sampled by serial filtration, total RNA is isolated and the rRNA removed. Subsequently, ds cDNA is synthesized from mRNA using random hexamers. The following cDNA sequencing is performed with a GS FLX Titanium Pyrosequencer. Finally, Metatranscriptomic data are analyzed in relation to environmental parameters using a novel data analyzing pipeline invented and developed in house.

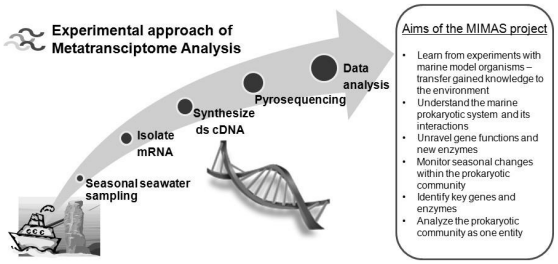

Fig. 2. Experimental approach of the Metatranscriptomic part in the MIMAS project

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