Combining ARISA and DGGE Approaches to Obtain Reproducible Quantitative Signatures of Microbial Community Shifts in the Soyang Aquatic Continuum

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TERRECO

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Targets and Tools

Bacteria and archaea as well as fungi are critical actors in ecosystems, since these organisms carry out the degradation of biomass and – in the case of bacteria or archaea – N-cycle transformations. Cyanobacteria and other bacterial phyla are also involved in C- and N-fixation. These microbial groups are also responsible for the production of toxins, which may severely damage certain economically important macro-organisms, may impact human populations, and can even disequilibrate systems. Developing tools to assay and characterize changes in microbial communities will permit us to observe and potentially monitor the relationships between microbes and ecosystem health at different scales. In Soyang Watershed, we are interested particularly in examining how microbial communities in the aquatic system are influenced or determined by exchange of groundwater with rivers, and input of materials to Soyang Lake. While focused on developing improved microbial community monitoring capacity, practical applications relate, on the one hand, to groundwater pollution that may occur due to culling and burial of diseased animals, and on the other hand, to dynamic changes in cyanobacterial growth that occur with water quality shifts during and after the summer monsoon.
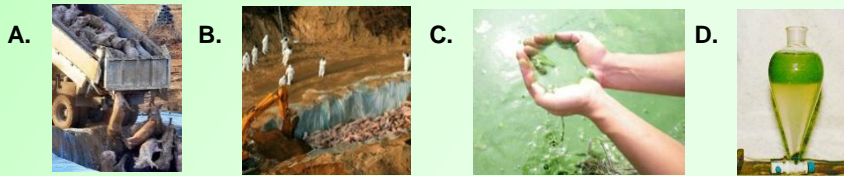
A.   **B.**   **C.**   **D.**

Fig 1. A. and B. One potential cause of shifts in aquatic microbial communities is large scale burial of diseased livestock http://www.piaberrend.org/pigs-buried-alive-in-south-korea-in-an-attempt-to-stop-the-spread-of-hoof-and-mouth-disease/ C. and D. An annual phenomenon now in Korean reservoirs is blooming of cyanobacteria and associated changes in other components of the reservoir microbial community during and after summer monsoon. Comprehensive molecular-ecological methodologies that monitor microbial communities help us better understand processes related to such events.

Molecular Diagnostic Tools That Will Be Combined

For monitoring the corresponding status and development of certain study sites, adequate molecular diagnostic tools should be selected for identifying and quantifying the members of such microbial communities. Denaturing Gradient Gel Electrophoresis (DGGE) and Automated Ribosomal Intergenic Spacer Analysis (ARISA) are commonly used in the context of ecological studies to estimate the number of bacterial and fungal taxa in terrestrial and aquatic samples and estimate their spatial and temporal dynamics. DGGE is a particular kind of electrophoresis which uses either a temperature or chemical gradient to denature the sample as it moves across an acrylamide gel or a capillary. In contrast, ARISA is a method using the length polymorphism of a defined gene as elements of a microbial sample signature. In the TERRECO context, DGGE is already used for groundwater studies in Haean Catchment and to characterize microbial communities in lakes and reservoirs (Fig. 2). ARISA has been applied at the DNA Analytics Laboratory of the University of Bayreuth to visualize the spatial and temporal dynamics of terrestrial microfungi in soils and plant tissues (Fig. 3 a–c).

Objectives

It is the aim of the envisaged project to use both approaches in parallel when analysing environmental aquatic and terrestrial samples for the occurrence patterns and seasonal dynamics of microbial communities in the Soyang aquatic system. Combining both techniques should lead to a much better resolution of the results. In addition, by introduction of ARISA (with the nrDNA ITS 1 gene for fungi and ITS1/2 for bacteria and archaea) detectable organisms are identifiable at least at domain level and via subsequent sequencing even at species level by using bands representing the taxon 'barcoding gene'.
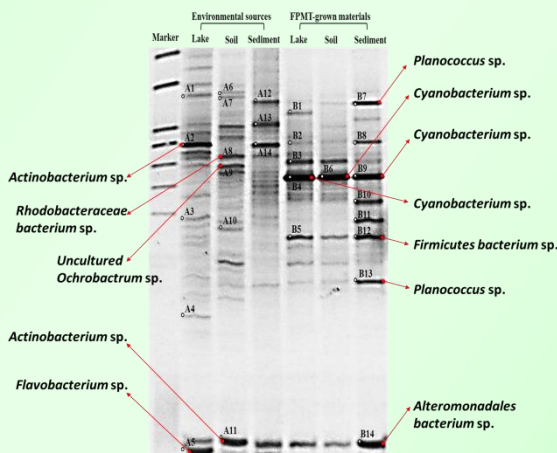
a

ANOSIM:	R	p (%)
plant - soil	0.712	< 0.01

△ plant   ○ soil

b

ANOSIM:	R	p (%)
● - ●	0.555	< 0.01
● - ●	0.940	< 0.01
● - ●	0.839	< 0.01

● Steigerwald   ● Solling   ● Hohe Warte

c

ANOSIM:	R	p (%)
Oe - Oa	0.278	0.2
Oe - A	0.966	0.02
Oe - B	0,792	< 0.01
Oa - A	0.232	5
Oa - B	0.365	0.07
A - B	0.300	0.9

△ Oe   ▽ Oa   ■ A   ■ B

Fig 2. PCR-DGGE profiles of environmental samples from Buus nuur soda lake in Mongolia

Planococcus sp.
Cyanobacterium sp.
Cyanobacterium sp.
Cyanobacterium sp.
Firmicutes bacterium sp.
Planococcus sp.
Alteromonadales bacterium sp.

Actinobacterium sp.
Rhodobacteraceae bacterium sp.
Uncultured Ochrobactrum sp.
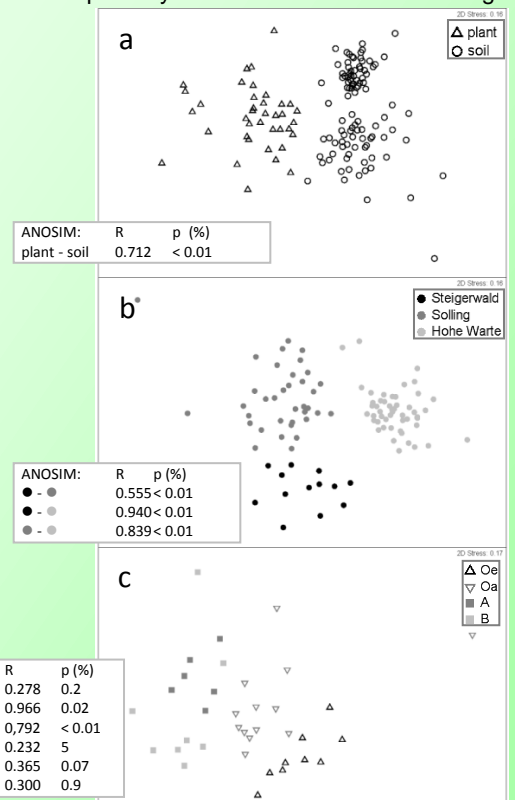Actinobacterium sp.
Flavobacterium sp.

Fig 3. ARISA NMDS distances of environmental samples from forest soils and trees in Germany