

Bacteria in Natural Forest Soils: Diversity and Search for Novel Genes

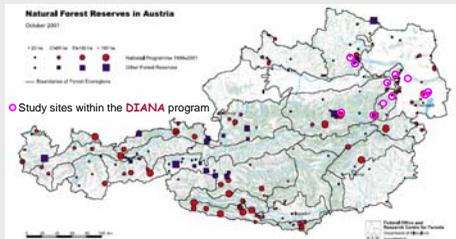
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Biodiversity of Natural Forests

Natural forests are composed of a natural vegetation which corresponds to specific site conditions. Although they have long been valued in studies of silviculture, these ecosystems have only recently gained recognition as sites of high biodiversity, which is due to their structural richness and to restricted anthropogenic disturbance. A network of 180 natural forest reserves has been established throughout Austria. A range of these forests have been surveyed with respect to plant species composition, soil chemical qualities, microbial biomass, microbially mediated nutrient transformation and soil biota. Thereby, distinct soil chemical and microbiological conditions have been found to prevail in contrasting forest types (Hackl et al. 2000, Hackl et al. 2004a, Hackl et al. 2004b, Hackl et al. 2005, Zechmeister-Boltenstern et al. 2005). In addition, 34 novel species of soil micro-, macro- and mesofauna have so far been discovered (Foissner et al. 2005, Pomorski et al. 2003, Lexer 2004).

Microbes such as bacteria and fungi are the most abundant and varied organisms on earth and exhibit a tremendous functional versatility. Our knowledge about the chemical capabilities of microbes is primarily based on those organisms which can be grown in defined culture media. The majority of soil bacteria, however, have so far resisted cultivation in the laboratory. Our objectives were (1) to assess the diversity of soil bacteria inhabiting various types of natural forest by applying nucleic acid based, cultivation independent methods; (2) and to relate bacterial community composition to soil chemistry and nutrient turnover, (3) with special emphasis being laid on bacteria involved in nitrogen cycling processes. (4) By use of a metagenomics approach, we strive to access the vast metabolic potential enclosed in the bacteria inhabiting the soil of the famous "Rothwald" indigenous forest.

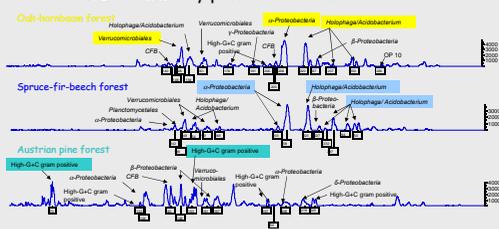


Biodiversity network "DIANA"
Soil Diversity In Austrian
Natural Forests
<http://bfw.ac.at/300/2197.html>

Diversity of Soil Bacteria in Austrian Natural Forests

Soil bacteria are key players in the recycling of elements, they stabilize soil structure and improve soil water retention. In natural forest soils, the microbial nutrient turnover has been shown to be related to soil type, climate and tree species composition (Hackl et al. 2004a). Our interest was centered on how the soil bacteria are organized in relation to nutrient turnover. The community structure of the soil bacteria was analyzed by T-RFLP (terminal restriction fragment length polymorphism) profiling and molecular cloning using the 16S rRNA gene as a phylogenetic marker.

T-RFLP community profiles of forest soil bacteria



T-RFLP community profiles of bacteria from oak, spruce-fir-beech and pine forest soils. T-RFLPs from the pine forest soil indicate that High-G+C gram positive bacteria are highly abundant, while in oak and spruce-fir-beech forest soils bacteria affiliated with the *Acidobacteria* seem to be highly prevalent.

By applying 16S rRNA gene analysis compositional differences in the bacterial communities inhabiting various natural forest soils were detected. These differences corresponded with microbiological and soil chemistry characteristics such as bacterial versus fungal biomass, soil pH and C to N ratio. Bacterial community composition apparently is related to specific environmental conditions which go along with various forest types. Our data represent important baseline information about bacterial diversity and composition in undisturbed forest ecosystems, which allows us to go forward with studying the effects of management practices or environmental pollution on soil bacterial communities.



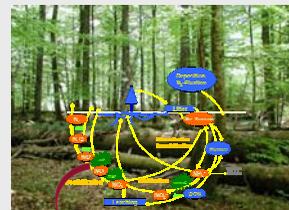
Oak-hornbeam forest
Quercus petraea Liebl., *Carpinus betulus* L., *Fagus sylvatica* L.

Spruce-fir-beech forest
F. sylvatica L., *Abies alba* Mill., *Picea abies* Karstn.

Austrian pine forest
Pinus nigra Amdt

Nitrifiers and Denitrifiers in the Rothwald Forest Soil

High nitrogen (N) input via atmospheric deposition may endanger biodiversity by causing nutrient imbalances known as "N saturation" of forest ecosystems. Through the activities of nitrifying and denitrifying bacteria, excess N may be transformed into nitrogen oxides, which act as potent greenhouse gases and are involved in the depletion of the stratospheric ozone layer. Previous studies imply that N is effectively retained through internal cycling in the Rothwald forest soil. Presently, the mechanisms and controlling factors of nitrogen loss and conservation are studied in this well-balanced forest by the analysis of functional genes encoding crucial enzymes in the nitrification and denitrification processes.



N-cycling in the Rothwald forest. The genes marked green are responsible for the production of key enzymes catalyzing the first steps of nitrification and denitrification and are studied via diversity and expression analysis.



T-RFLP profile of *nirK*-genes reflecting the community composition of nitrite reducing bacteria in the Rothwald forest soil.

Metagenomic Analysis of the Rothwald Forest Soil

Biodiversity has long served as a basis for the development of novel compounds for the use in biotechnology, agriculture and pharmacology. Due to the low culturability of microorganisms from natural environments, only a small fraction of the huge genetic and metabolic diversity encountered in the microbial world has so far been explored. New techniques involving the recovery and direct cloning of large fragments of environmental microbial DNA (termed the metagenome) enable to screen microbial communities for novel genes without the need of prior cultivation (e.g. Rondon et al. 1999, Quaiser et al. 2002). Thus, through the metagenomics approach access is provided to the vast metabolic potential enclosed in the diverse microbial communities inhabiting the Rothwald forest soil. Our primary aim is to screen the Rothwald metagenome for novel nitrite reductase genes involved in bacterial denitrification. However, metagenomic libraries represent a rich pool of novel biostructures, including biotechnologically relevant enzymes and metabolites.



Overview of the process for preparing a metagenomic "fosmid" library (EPICENTRE Biotechnologies).

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