

Endophyte Research at the AIT

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INTRODUCTION

The term endophyte refers to interior colonization of plants by microorganisms that usually do not cause damage to the host and live inside the plant without exhibiting pathogenicity. This definition includes organisms that "fluctuate" during their endophytic phase between internal (endophytic) and external (epiphytic or rhizosphere) areas.

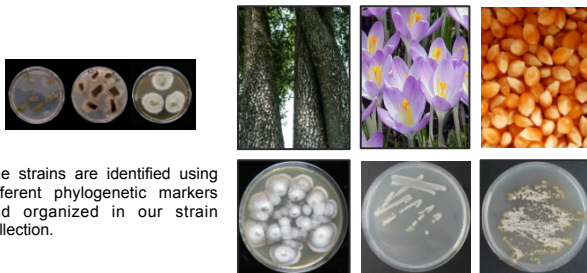
For a long time endophytic bacteria have been regarded as contaminants from incomplete surface sterilization. Extensive analysis (cultivation-based and cultivation-independent) of endophytic bacteria in the last decade allowed the identification of bacterial endophytes in both monocotyledonous and dicotyledonous plants. In general, plants host 50 to 100 different species representing different percentages of the whole population, but typically only approximately ten will be dominant.

The endophytic community of only a few plants has been studied in more detail and that contain a range of non-pathogenic bacteria belonging to various phylogenetic groups. Most dominant seem to be *Proteobacteria*, the low and high GC Gram-positives, the *Cytophaga/Flavobacterium/Bacteroidetes* phylum and the *Holophaga/Acidobacterium*.



ENDOPHYTE STRAIN COLLECTION

So far, more than 1000 bacterial strains have been isolated at AIT from various plant species, ranging from woody tree species to wild flowers.



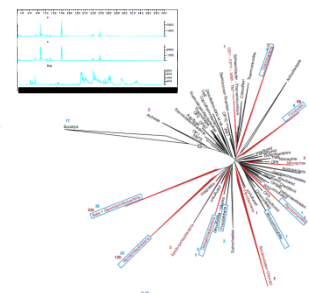
The strains are identified using different phylogenetic markers and organized in our strain collection.

CULTIVATION-INDEPENDENT ANALYSIS OF ENDOPHYTES

The majority of bacteria is not accessible by cultivation and therefore, cultured bacterial species represent only a minor fraction of the existing diversity.

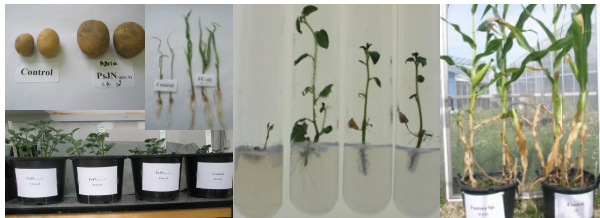
We analyse mixed environmental DNA and RNA and employ DNA-fingerprint techniques as well as cloning and sequencing of various phylogenetic marker genes to assess the diversity of uncultured endophytes.

We construct metagenomic libraries in various bacterial hosts to get access to the gene pool of uncultured endophytes.



BENEFICIAL EFFECTS OF ENDOPHYTES

Host-plant/endophyte interactions are often considered as beneficial – the bacteria gain nutrients and a protected niche to occupy, whereas the host benefits from potential plant growth promoting effects, such as plant hormone production, improvement of nutrient uptake, increased stress tolerance, antagonistic effects against plant pathogens and the induction of senescence.

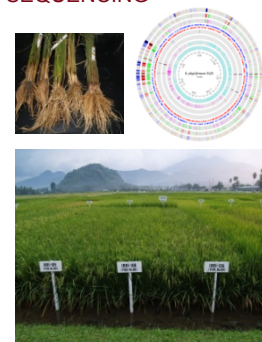


GENOME AND METAGENOME SEQUENCING

Although the existence of diverse endophytic communities in individual plants has been known for years, their genetic is mostly unknown.

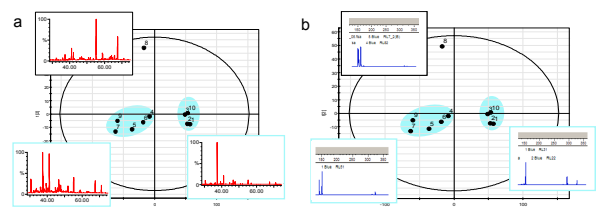
To understand and manipulate their contribution to plant development, it is important to determine metabolic processes, adaptations and beneficial characters.

We therefore do comparative genome and metagenome analysis. Based on the findings we set-up genetic experiments.



METABOLITE LIBRARIES

We find strong correlations between plant secondary metabolites and endophyte community composition indicating interaction between endophytes and plant physiology. Together with high plant physiological activity associated endophytes may also exhibit high metabolic activity. We therefore analyze the metabolic capacities of endophytes resulting in an endophyte-metabolite library.

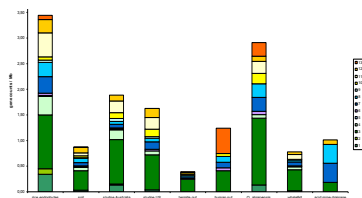
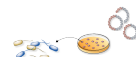


PLS analysis of plant drimane sesquiterpene (a) and total 16S rDNA-T-RFLP (b) pattern in leaves of ten individual *Warburgia ugangandensis* trees grown in Kenya.

MOLECULAR SCREENINGS

Endophytes encounter in plants a range of complex organic compounds that are not found in soils or other environments and so it's not surprising that we find an outstanding high number of genes for e.g. cellulases, pectinases, ring-cleaving dioxygenases in endophytes.

We construct genomic and metagenomic (small and large insert) libraries and develop molecular screens to exploit the metabolic and enzymatic versatility of endophytes.



Comparative metagenomic analysis. Counts on pfam domains for the degradation of aromatic compounds

SELECTED PUBLICATIONS

- Sessitsch, A., Reiter, B., Pfeifer, U., and E. Wilhelm. 2002. Cultivation-independent population analysis of bacterial endophytes in three potato varieties based on eubacterial and *Actinomyces*-specific PCR of 16S rRNA genes. *FEMS Microb. Ecol.*, 39:23-32.
- Reiter, B., U. Pfeifer, H. Schwab, and A. Sessitsch. 2002. Response of endophytic bacterial communities in potato plants to infection with *Erwinia carotovora* subsp. *atroseptica*. *Appl. Environ. Microbiol.* 68:2261-2268.
- Reiter, B., Bürgmann, H., Burg, K., and A. Sessitsch. 2003b. Endophytic nifH diversity in African sweetpotato. *Can. J. Microbiol.* 49: 549-555.
- Sessitsch, A., T. Coenye, A.V. Sturz, P. Vandamme, E. Ait Barka, J.F. Salles, J.D. van Elsas, D. Faure, B. Reiter, B.R. Glöck, G. Wang-Pruski, and J. Nowak. 2005. *Burkholderia phytofirmans* sp. nov., a novel plant-associated bacterium with plant beneficial properties. *Int. J. Syst. Evol. Bacteriol.*, 55:1187-1192.
- Reiter, B., and A. Sessitsch. 2006. Bacterial endophytes of the wildflower *Crocus albuliflorus* analyzed by characterization of isolates and by a cultivation-independent approach. *Can. J. Microbiol.* 52: 140-149.
- Compant, S., H. Kaplan, A. Sessitsch, J. Nowak, E. Ait Barka and C. Clement. 2008. Endophytic colonization of *Vitis vinifera* L. by *Burkholderia phytofirmans* strain PsJN: from the rhizosphere to inflorescence tissues. *FEMS Microbiol.Ecol.* 63:84-93.
- Rasche, F., T. Lueders, M. Schloter, S. Schaefer, F. Buegger, A. Gättinger, R.C. Hood-Nowotny and A. Sessitsch. 2009. DNA-based stable isotope probing enables the identification of active bacterial endophytes in potatoes. *New Phytologist* 181: 802-807.
- Compant, S., M. van der Heijden and A. Sessitsch. 2010. Climate change effects on beneficial plant-microbe interactions. *FEMS Microbiol. Ecol.* 73:197-214.
- Compant, S., C. Clement and A. Sessitsch. 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biol. Biochem.* 42:669-678.