Characterisation, control, and utilisation of bacterial endophytes in in-vitro cultures of *Prunus avium* L.

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Project aims

Plant material

Prunus avium is cultivated as a fast growing hardwood for the production of high quality furniture. Fast-growing trees and such with a straight growth have the highest value on the market. To achieve these characteristics single trees with a good habitus are selected and propagated as in-vitro clones.

Challenge

During propagation, rooting and acclimatisation, large-scale losses of plantlets have been observed. It is expected, that this is caused by the presence of endophytes.

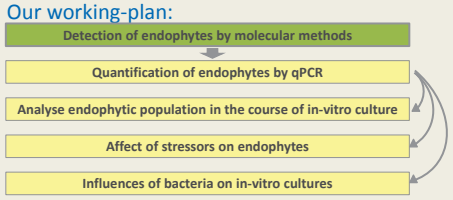
Source (C+D): Booklet on *Prunus avium*, silvaSELECT Gehölze GmbH

Prunus avium in-vitro culture (A, B), pottet plants after acclimatisation (C) and 19 year old tree (D).

Project background

We want to investigate the interaction between *Prunus avium* (wild cherry) in-vitro plantlets and bacterial endophytes and try to differentiate between bacteria with beneficial, neutral or negative properties for the plant. This knowledge will be used to balance the endophytic population and to enhance positive bacteria.

Our working-plan:

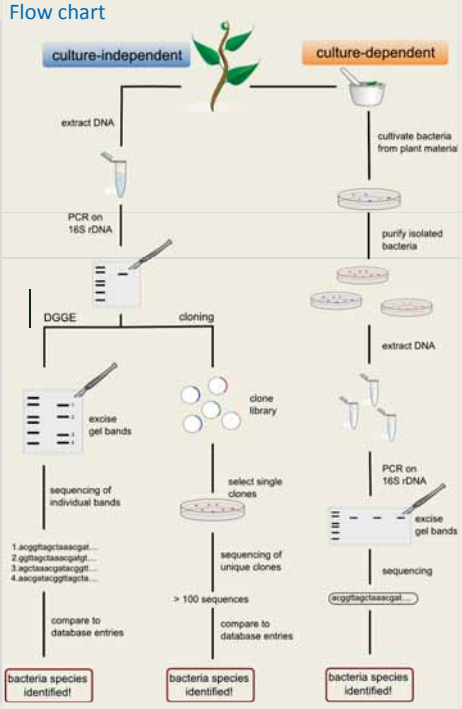


Detection of endophytes

Two strategies can be used to analyse endophytes:

- Culture-dependent approach
- Culture-independent approach

Flow chart



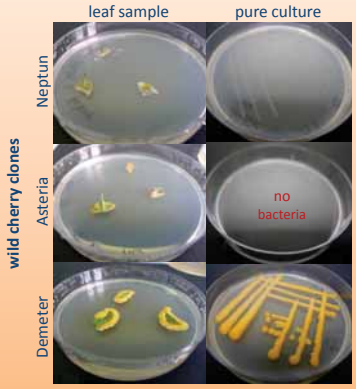
Culture-dependent approach

- Bacteria were isolated from crushed plant material (serial dilutions) or from leaf- and stem-segments placed on solid medium.
- Six *Prunus avium* clones with good, medium and poor propagation success were used.
- Four clones showed bacterial growth after 2 weeks on bacteria screening medium 523.
- A swap was taken and streaked on new agar twice to receive a pure culture.

Further steps:

- Sequencing of amplified fragments.
- Identification of bacteria species or genera.

Isolation of bacteria from leaf segments



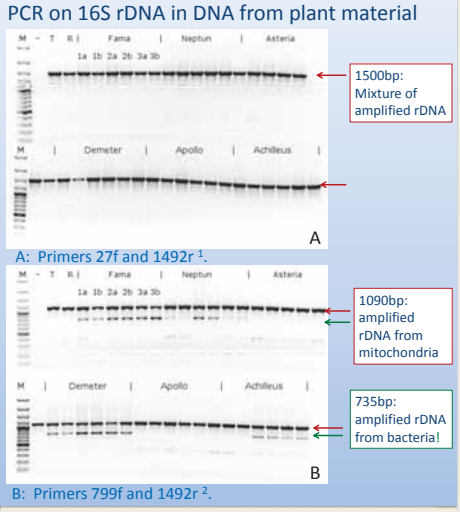
Culture-independent approach

- Bacterial DNA was extracted from in-vitro plantlets.
- A PCR on 16S rDNA was conducted with three plants of each plant clone, two samples were taken per plant. Two primer pairs were used (see figure A+B).
- PCR products were excised from an agarose gel and purified.

Further steps:

- PCR-DGGE and sequencing to identify species or genera.
- Clone library and sequencing to identify species or genera.

PCR on 16S rDNA in DNA from plant material



A: Primers 27f and 1492r [1].

B: Primers 799f and 1492r [2].

Outlook

- Sequencing of the PCR-amplificates will reveal the species identity of endophytes in the *Prunus avium* in-vitro cultures.
- Establishment of qPCR protocols for bacteria of interest will enable quantification of specific species densities in different plant organs under different cultivation conditions. This will allow to correlate increasing / decreasing plant quality with specific changes in the endophyte population.
- Influences of chemical and physical factors on wild cherry in-vitro culture will be analyzed and used to enhance the quality of plant material.

The authors thank the German Federal Ministry of Economics and Technology for financial support within the program ZIM KF and the Institute of Plant Culture for providing the plant material for our research.

References: (1) Thomas (2008) Ubiquitous presence of normally non-culturable endophytic bacteria in field shoot-tips of banana and their gradual activation to quiescent cultivable form in tissue cultures. Plant Cell, Tissue and Organ Culture, 93: 39-54

(2) Chelius and Triplett (2001) The Diversity of Archaea and Bacteria in Association with the Roots of *Zea mays* L. Microbial Ecology 41: 252-263