Establishment of a fingerprinting method for analysis of fungal communities on grapevine

Christian Kraus[1], Michael Fischer[1], Ralf Vögele[2]
[1] Julius Kühn-Institut, Institute for Plant protection in Fruit Crops and Viticulture, Siebeldingen
[2] University of Hohenheim, Department of Phytopathology, Hohenheim
Email of corresponding author: christian.kraus@jki.bund.de

Since the last two decades a number of ´fingerprinting´ methods have been developed to analyze microbial communities and their dynamics, including Terminal Restriction Fragment Length Polymorphism (T-RFLP), Length Heterogeneity-Polymerase Chain Reaction (LH-PCR) and Automated Ribosomal Intergenic Spacer Analysis (ARISA). Because the latter provides a quick and cheap way together with high accuracy, we have chosen this method to investigate the fungal communities on grapevine, wood, leaves and berries.

As a first step in ARISA, a PCR approach amplifies the internal transcribed spacer (ITS) region of fungal DNA samples. As the ITS region represents non-coding DNA, it is extremely variable in nucleotide sequence and length. One of the primers used in the PCR is labeled with a fluorescent dye, which can be detected as a peak in an electropherogram. Because of the length variability of the ITS region, each fungal species ideally forms an individual peak in the electropherogram. Furthermore the height of the peak gives a hint about the abundance of the fungus within the community.

To assign the peaks to the corresponding fungus, a database has to be created, containing the peak position (correlating with the size of the amplicon) for each fungal species.

Taken together the ARISA provides a valuable tool which gives important information not only about the fungal diversity within a given sample but also about the quantity of each fungus.
This technique shall help us to study the composition of fungal communities on grapevine and how they change compared to different environmental conditions in relation to cultivars as well as training and plant protection regimes.