

Phytotyper - Identification of phytopathogenic microorganisms by MALDI Biotyper

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Bacterial plant diseases are a major concern, therefore prevention and control of epidemics relies on fast and reliable diagnostics. One diagnostic system for bacterial species identification, which became well established in clinical microbiology over the last years due to short processing time, high throughput, and universality of the protocol, is MALDI Biotyper.

The MALDI-TOF MS based system generates mass spectra of the proteins from either whole bacterial cells or cell extracts. The protein peak patterns originate from the most abundant proteins in the cells and are specific for the respective bacteria. Taxonomic identification of microorganisms is possible by matching the generated peak pattern against the peak lists of spectra from known bacterial isolates. Depending on the Taxa and the number of reference spectra, resolution can be at the genus-, group-, or species-level. A database containing around 9,600 reference spectra is provided by the Biotyper manufacturer. However, in the manufacturer's database coverage of microorganisms in phytomedicine context is low and more random, than practice-oriented.

Therefore, the aim of the BMEL-funded Phytotyper project is to improve MALDI-TOF MS based diagnostic methods for plant-health-related bacteria. The first part of the project includes the build-up of a contextual reference database with coverage of relevant plant pathogenic bacteria and non-pathogenic accompanying microorganisms from diverse samples and species for facilitated diagnostics. Simultaneously, information on microbial diversity in different plant production systems will be obtained. Furthermore, for heterogeneous species like the *Pseudomonas syringae* group, for which with the manufacturer's database assignment is rarely possible on species-, let alone on pathovar-level, higher resolution will be achieved by higher coverage in the database, as higher coverage in the database leads to higher resolution in the species identification.

The advantage of the Biotyper technique is the possible detection of all cultivable microorganisms on a plant sample. This facilitates diagnostics for samples in which saprophytic bacteria mask the actual pathogen, or in which conventional diagnostic methods cannot identify a distinct pathogen. Also, isolates for which no reference is available can be grouped according to their similarity and, without further characterization a reference can be created. Thus, these unidentified isolates can already be used for diagnostic purposes. In addition, relevant yet unidentified isolates will be further characterized by either Sanger sequencing or multilocus sequence analysis (MLSA).

To date, our database consists of 486 entries from six different plant samples and several microbiological isolates from routine diagnostics. For half of these entries identification was possible at least to the genus level.