# MALDI-TOF mass spectrometry for the diagnosis of plant pathogenic bacteria

Matrix-assisted laser desorption/ionization - time-of-flight mass-spectrometry (MALDI-TOF MS) is a technique that allows the mass of molecules to be determined very precisely. It can be applied to pure molecules as well as complex mixtures. The technology can be used for the identification of pathogens by comparing mass spectra of the sample to databases of reference spectra. The quality of the reference database is crucial for a reliable identification. If databases for clinical microbiology allow rapid and reliable diagnosis of many human pathogens, this is not the case for plant health because plant pathogenic bacteria have not been widely analysed with MALDI-TOF MS and their reference spectra are largely absent from databases.
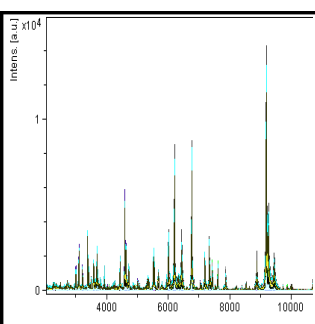
**One of the objectives of the Euphresco project MALD-ID was to characterize a number of plant pathogenic bacteria in order to produce reference spectra and contribute to the development of reliable databases. Another objective was to evaluate the use of the MALDI-TOF MS for the diagnosis of selected bacterial genera, species and subspecies. The project gathered five European organizations: ZHAW (CH), JKI (DE), INRAE (FR), NVWA (NL) and NIB (SI).**

During the project, reference spectra were obtained for strains that represent the diversity of a selection of genera which are important for plant health: *Clavibacter*, *Curtobacterium*, *Erwinia*, *Pantoea*, *Pectobacterium*, *Pseudomonas*, and *Xylophilus*.

As the method is based on the determination of the mass of (ribosomal) proteins of the cell, the conditions used to grow the bacteria have an impact on the results of the test. The project partners developed standardized procedures for bacterial culture and sample preparation for the abovementioned genera in order to maximize the performance of the test. The procedures will be part of an EPPO Standard, currently under development. The reference spectra produced by the partners will be made available at the end of the project.

A test performance study was organized to validate the use of MALDI-TOF MS for bacterial identification on the basis of the newly generated spectra.

The test performance study showed that MALDI-TOF MS allows correct identification of bacteria at genus level with high sensitivity and specificity. The performance of MALDI-TOF MS for the identification of bacteria at species level is variable. *Pantoea* spp. can be identified reliably, but for other genera such as *Pseudomonas* or *Pectobacterium*, MALDI-TOF MS is not always able to discriminate between the species. It was also demonstrated that the technique is not precise enough to discriminate at sub-species or pathovar level. In plant-pathology, identification is often needed at subspecies level. For instance, *Pantoea stewartii* subsp. *stewartii* is a quarantine bacterium in Europe, while *Pantoea stewartii* subsp. *indologenes* is not. This means that MALDI-TOF MS is not precise enough to be used for the identification of regulated bacteria, but it is a powerful, reliable and rapid tool for screening, as it allows the genus of an isolate to be rapidly determined, thus facilitating the choice of the best identification test.

Project ID: Rapid identification of plant health related bacteria by MALDI-TOF mass spectrometry (MALD-ID).