

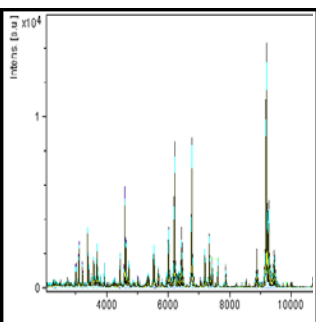


# Euphresco Success story

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## MALDI-TOF mass spectrometry for plant pest diagnostics: the case of the accurate identification of *Acidovorax citrulli* isolates, including their grouping

Matrix-assisted laser desorption/ionization and time-of-flight mass-spectrometry (MALDI-TOF MS) is an emerging technology exhibiting promising results for the identification of bacterial plant pathogens. In clinical microbiology, this technology is already successfully used. The limited use of MALDI-TOF MS for the identification of plant pathogenic bacteria has been mainly attributed to the lack of comprehensive and commercially available databases on reference microorganisms. However, creation of in-house databases supplementing the commercial databases has been proven to be highly adequate for the identification of various plant pests which are currently under-represented in the commercially available databases.



**Using *Acidovorax citrulli* as a model, the Euphresco project DIP-ACIT aims to demonstrate the potential of MALDI-TOF MS for an accurate and rapid identification, including grouping, of *A. citrulli* isolates.**

There is a well documented distinction of virulence among isolates of *A. citrulli*. Based on genetic and pathogenic assessment, *A. citrulli* is divided into 2 groups. While group I isolates are moderately aggressive on a range of cucurbit hosts including watermelon, group II isolates are highly aggressive on watermelon but moderately aggressive on non-watermelon hosts.

In order to cope with the lack of a commercially available database, an in-house database supplementing the commercial database and covering the known genetic diversity inside this bacterial species was developed and evaluated. Six reference isolates of *A. citrulli*, one of *Acidovorax cattleya* and one of *Acidovorax konjaci* were selected and the protein mass fingerprints of each reference isolate were used to generate Mass Spectral Profiles (MSPs) by identifying their mass peaks. For each reference isolate, a MSP was generated through the accumulation of 8 technical replicates of the same spectrum and each MSP was checked for specificity before entry into the in-house database.



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The in-house database ready, isolates of *A. citrulli* and related species were prepared for MALDI-TOF MS analysis by either directly transferring bacterial cells from NA (Nutrient Agar) plates (direct transfer method) or after their prior treatment with formic acid (direct formic acid method). The spectra of all individual isolates were analyzed against the in-house database, in combination with the commercial database. Both methodologies, the direct transfer method and the direct formic acid method, performed equally well. The in-house database was shown to be specific for *A. citrulli* and has exhibited a great potential in directly grouping the individual isolates of *A. citrulli*. Additionally, this study clearly highlighted the potential and the simplicity of MALDI-TOF MS for a rapid and accurate identification of *A. citrulli*, just in a few seconds.

Further validation of the method will be obtained using a number of *A. citrulli* isolates (plant and seed extracts and pure cultures) prepared for the interlaboratory comparison (currently ongoing) organized by the University of Modena and Reggio Emilia and involving 10 international laboratories.

Project ID: Consensus detection and identification protocol for *Acidovorax citrulli* on cucurbit seeds (DIP-ACIT).