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KTI Medtech Event 2010

Project Nr. 11225.1 PFLS-LS

Starting date: July 2010, Duration of project: 3 years

Development and validation of a MALDI-ToF MS microbial database for rapid identification of public-, animal-, and plant-health relevant environmental bacteria.

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Main industrial partner: **Dr. Guido Vogel**; MABRITEC AG (Gesellschaft für massenbasierte rasche Identifikationstechnologien), 4125 Riehen.
Co-applicants: **Dr. Brion Duffy**, **Dr. Fabio Rezzonico**; Agroscope Changins-Wädenswil, Postfach, 8820 Wädenswil.

Project summary

MALDI-TOF Mass Spectrometry is an emerging tool for routine identification of microorganisms. While delivering results comparable to DNA sequencing, MALDI-ToF MS is faster, easier to carry out and more cost effective. Strain-specific mass spectra can be produced within minutes starting from a bacterial colony growing on agar, but reliable identification depends upon a validated and comprehensive database of peptide mass fingerprints. These are currently only available for selected human pathogenic bacteria, therefore expansion to broader areas of microbiology present a potentially lucrative market niche. Without a reference database that includes environmental bacteria reference spectra, there is no application in plant-animal health, and only restricted application in human health diagnostics. The aim of this project is to develop and validate a marketable database of MALDI-ToF MS data for routine application in plant, animal and wider public health fields. With the database produced in this KTI project, MALDI-ToF MS can be used to simplify diagnostics and improve the implementation of control/therapy strategies.

Utilization for the identification of *Pantoea* species

We illustrate here as example the results obtained using MALDI-ToF MS for the identification of *Pantoea*, a bacterial genus including both clinical and environmental strains (some of them with potential biocontrol applications), that emerged after countless taxonomical reorganizations from the *Enterobacter agglomerans-Erwinia herbicola* complex, which resulted in still unsettled nomenclature problems, frequent misidentification of *Pantoea agglomerans* as opportunistic human pathogen and erroneous classification of several strains.

Bacterial strains A set of strains retrieved from culture collections as *P. agglomerans* (or under the old basonym *Enterobacter agglomerans)* were chosen for the analysis **(Fig. 1)**. These strains included specimens from several culture collections (ATCC/LMG/CIP/CECT), clinical isolates, environmental isolates and strains with known biocontrol activity against fire blight on apple trees. Bacteria were grown on LB agar at 28°C for 24-48 hours. For all strains, sequencing of the *gyrB* gene was performed as comparison to confirm their identity prior to MALDI-ToF analysis.

Spectral analysis Generated mass spectra were analyzed with the SARAMIS™ (Spectral ARchive And Microbial Identification System) application from Anagnostec. A taxonomic tree (single-link clustering) was built using the peak pattern of all analyzed strains and compared to a phylogenetic tree (Minimum Evolution) of the *gyrB* gene **(Fig. 2)** . Species-specific biomarkers were identified and used for the creation of a SuperSpectrum™ which can then be utilized for the recognition of *P. agglomerans* isolates.

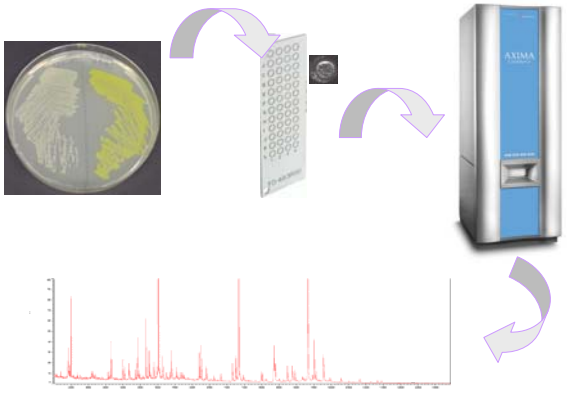

Figure 1 Workflow of MALDI-ToF/SARAMIS™ analysis. A strain-specific mass spectrum can be produced within two minutes starting from a bacterial colony growing on agar plate. Cells are put directly on a steel slide with a loop and embedded in 0.5 µl of matrix (e.g., 10 mg/ml 2,5-dihydroxybenzoic acid with 0.03% trifluoroacetic acid). Preparation is completed within seconds after samples are air-dried. Slides are then analyzed with a minimum of 50 laser shots per sample to generate mass spectra and a single-link clustering taxonomic tree can be built using SARAMIS™. Identification of a sample is achieved by comparison to the SuperSpectra™ present in the database or new SuperSpectra™ can be generated by choosing specific mass signals shared by all the representatives of a species.

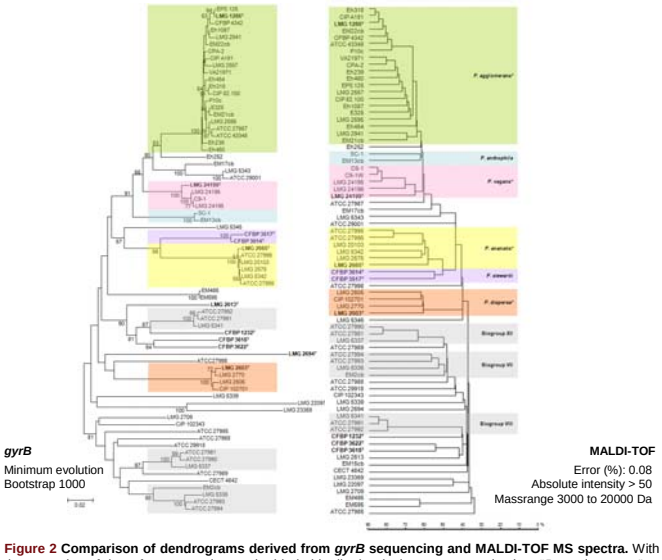

Figure 2 Comparison of dendrograms derived from *gyrB* sequencing and MALDI-TOF MS spectra. With the exception of the reference strains marked in bold, all other isolates were received as "*P. agglomerans*" or "*E. agglomerans*". The main clusters obtained in the tree generated with MALDI-ToF data correspond largely to those obtained by sequencing the *gyrB* gene. Both methods were equally proficient in identyfing *P. agglomerans* from other species and in recognizing previously misidentified stains.

Reference Rezzonico *et al.* (2010) Application of whole-cell matrix-assisted laser desorption ionization-time of flight mass spectrometry for rapid identification and clustering analysis of *Pantoea* species*. Appl. Environ. Microbiol.* **76:**4497-4509.

Perspectives

In the first phase a series of relevant type strains from different culture collections will be analyzed by MALDI-ToF MS. This series should provide a directly usable core database that will be expanded in following phases by adding strains from the applicants' collections after their identification and typing by sequencing specific genes (e.g., *gyrB*, 16S rDNA) or with other gold standard methods for species identification. This will result in the generation of specific biomarker sets (SuperSpectra™) from each isolated species-type for their use as identifiers. We have successfully completed the proof-of-principle pilot project and demonstrated that MALDI-TOF allows the identification of *Pantoea* down to the species level. We are currently starting a taxa-by-taxa approach that will ultimately allow us to include all projected species in the database. Preliminary results with plant pathogenic *Xanthomonas* spp. suggest that MALDI-ToF is able to discriminate strains even to the subspecies (pathovar) level.

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