

1. Content of the 'Topic Description' document

1.1. Topic area

Diagnostics

1.2. Links to the Euphresco Strategic Research Agenda

The topic addresses the following objective(s) of the 2017-2022 Euphresco Strategic Research Agenda:

Objective 2017-I-2.2: to contribute to databases for plant pests identification and diagnostics

1.3. Topic title

Rapid identification of plant-health related bacteria by MALDI-TOF mass spectrometry

1.4. Description of the problem the research should solve

MALDI-TOF MS (matrix assisted laser desorption/ionization – time of light mass spectrometry) has been a useful tool for rapid microbial identification in clinical microbiology. Using this technique, one detects and characterizes single charged analyte ions. This results in a mass to charge (m/z) fingerprint of whole cell proteins which is specific for a species, subspecies or strain of bacteria. The main peaks in the fingerprint generally correspond to the abundantly expressed ribosomal proteins. Advantages of this method are low cost of sample preparation and rapid experimental procedure. Identification relies on the existence of reliable databases. Plant-associated bacteria have not been widely evaluated with the MALDI-TOF MS technique and profiles of reference strains of these bacteria are largely absent from commercially available identification databases. At this moment, this limits the application of this technology in plant pathology.

Recent studies have shown that MALDI-TOF MS can be a useful technique for identification of plant pathogenic bacteria. For example, the technique has sufficient discriminatory power to distinguish members of the genus *Clavibacter* from the related *Curtobacterium* genus, and to distinguish the different subspecies of *Clavibacter michiganensis* from each other (Zaluga *et al.* 2011). As described in a recently published Euphresco success story, the Euphresco project DIP-ACIT aims to demonstrate the potential of MALDI-TOF MS for accurate and rapid identification of group I and group II isolates of *Acidovorax citrulli*. In addition, validation data demonstrated that an in-house database of Mass Spectra Profiles - supplementing the commercial database - generated by MALDI-TOF MS was very specific, resulting in the rapid and accurate identification of *Ralstonia solanacearum* (phylotype II), *Ralstonia pseudosolanacearum* (phylotype I) and *R. pseudosolanacearum* (phylotype III) (Van de Bilt *et al.*, accepted *EJPP*).

The limitations of having insufficient profiles of reference strains of plant-associated bacteria in commercially available identification databases can be overcome by the creation of in-house databases supplementing commercial databases. The aim of this project is (1) to identify missing data/needs for increased resolution of plant pathogens in the current database; (2) to characterize a number of plant-health relevant bacteria to initiate development of reliable database, and (3) to perform interlaboratory comparisons/parallel analysis to check for potential reproducibility/data exchange issues. At some point, validation of selected database entries would be required (especially of interest for heterogeneous bacteria). Potentially an EPPO protocol on MALDI-TOF identification / validation guidelines could be developed.

1.5. Description of the expected results

- A list of the main gaps in the current databases for identification of plant pathogenic bacteria
- New entries to these databases
- Parallel analysis to better understand potential data exchange issues

1.6. Beneficiaries of this research product

- NPPOs
- Diagnostic laboratories

1.7. Research funders and research contribution/ distribution

Funding organisation	Research activity and researchers involved
<p>1. National Institute for Agronomic Research, France</p> <p>Jean-Pierre Rossi jean-pierre.rossi@inra.fr</p>	<p>-Project coordination; -Scientific contribution to be detailed</p> <p>Contact person: Perrine Portier E.mail: perrine.portier@angers.inra.fr</p>
<p>2. Netherlands Food and Consumer Product Safety Authority (NVWA), the Netherlands</p> <p>Martijn Schenk M.Schenk1@nvwa.nl</p>	<p>-Access to a Bruker linear-mode apparatus from the microflex series (participation in interlaboratory comparisons); -Application of MALDI-TOF for identification of bacteria from e.g. the genera <i>Ralstonia</i> and <i>Clavibacter</i></p> <p>Contact person: Maria Bergsma-Vlami E.mail : m.vlami@nvwa.nl</p>
<p>3. All-Russian Plant Quarantine Centre, Russia</p> <p>Natalia Sherokolava natalia_sh@mail.ru</p>	<p>-Contribution to be detailed</p> <p>Contact person: Konstantin Kornev Email address: konstantin.kornev@gmail.com</p>
<p>4. Ministry of Agriculture Forestry and Food, Slovenia</p> <p>Erika Oresek Erika.Oresek@gov.si</p>	<p>-Access to a Bruker linear-mode apparatus from the microflex series (participation in interlaboratory comparisons); -Application of MALDI-TOF for identification of bacteria of e.g. <i>Pantoea</i>, <i>Dickeya</i> and Xanthomonadaceae</p> <p>Contact person: Tanja Dreo E.mail : tanja.dreo@nib.si</p>
<p>5. Ministry of Agriculture Forestry and Food, Slovenia</p> <p>Erika Oresek Erika.Oresek@gov.si</p>	<p>-Access to a Bruker linear-mode apparatus from the microflex series; -Application of MALDI-TOF for identification of non-quarantine plant bacteria such as <i>Pseudomonas syringae</i> complex, <i>Pseudomonas fluorescens</i> complex and Xanthomonadaceae</p> <p>Contact person: Janja Lamovšek</p>

<p>6. Zurich University of Applied Sciences, Switzerland</p> <p>Fabio Rezzonico fabio.rezzonico@zhaw.ch</p>	<p>E.mail : anja.lamovsek@kis.si</p> <p>-Access to a Shimadzu (bioMérieux) linear-mode apparatus from the Axima Performance series; -Application of MALDI-TOF for identification of bacteria of various genera (mainly <i>Ralstonia</i>, <i>Xanthomonas</i> and possibly <i>Erwinia</i>)</p> <p>Contact person: Joël Pothier E.mail : joel.pothier@zhaw.ch</p>
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1.8. Research project partnership outside Euphresco

Euphresco funding ensures a certain level of transnational collaboration among Euphresco member countries. It is possible, if the funding consortium is interested, to contact funding organisations or research groups outside the geographical area covered by Euphresco members. The Euphresco coordinator could advertise the research topic in order to have an enlarged collaboration. If funders are interested in this possibility, please check the case below:

The funding consortium of the topic mentioned in section 1.2 requires that the topic is advertised outside the Euphresco network

Information to define the profile of sought partners could be useful (but not mandatory): country/region (if there are preferences), skills/expertise required, etc.

1.9. Any other relevant information on content

Expressions of interest were received from:

Cledir Santos cledir.santos@ufrontera.cl from Universidad de la Frontera, Chile

Murugaiyan Jayaseelan Jayaseelan.Murugaiyan@fu-berlin.de from Freie Universität Berlin, Germany

Nelson Lima, nelson@ie.uminho.pt from the University of Minho, Portugal

2. Eupresco management aspects of the project

2.1. Indication of the topic budget

Funding organisation ^a	Mechanism ^b	Total Budget ^c
1. INRA (FR)	NC	€ 20 000
2. NVWA (NL)	NC	€ 10 000
3. FGBU-VNIIEKR (RU)	NC	€ 10 000
4. MKGP (SI)	NC	€ 22 000
5. ZHAW (CH)	NC	€ tbc
total		€

2.2. Expected duration of the project (only for non-competitive topics)

24/36 months

2.3. Identification of project coordinator

Has the research project coordinator been identified?

Yes

No

2.4. Any other relevant information on topic organisation and management

^a First member is project coordinator. A minimum of two partners are necessary for each proposal. Add lines as needed.

^b Please indicate the preferred mechanism (e.g. real pot RP; virtual pot VP; non-competitive NC), or several mechanisms if there is flexibility.

^c Optional, as this amount can still change in the next phase. In-kind contribution should also be indicated in this column.