European Network for the durable exploitation of crop protection strategies

IA3 Activity: *Human resource exchange*

ENDURE – PhD Scholarship

Final activity report

(The form has to be filled in and sent to the activity leader – message should be sent to his p.a. elisa.scanzi@ibaf.cnr.it – within 15 days after the end of the visit)

Topic of the visit

1. Information about researcher and sending partner

Name and surname: Małgorzata Lisowska

Professional status: PhD student

Sending partner:

Institute/Department/Research Unit:
Plant Breeding and Acclimatization Institute/ Department of Plant Pathology/ Laboratory of quarantine diseases.
Address: Radzików, 05-870 Błonie.

E-mail and phone number of the researcher: m.lisowska@ihar.edu.pl

Supervisor name*:Edward Arseniuk

Supervisor e-mail*: e.arseniuk@ihar.edu.pl

Supervisor phone number*: (+48 22) 725 30 95

*Supervisor information only for PhD student, post-doc and junior researchers

2. Information about hosting partner

Hosting partner:
Plant Research International/

Institute/Department/Research Unit: Biointeractions and Plant Health

Address: Droevendaalsesteeg 1, 6708 PB Wageningen The Netherlands

Supervisor name*: Jan van der Wolf

Supervisor e-mail*: Jan.vanderwolf@wur.nl

Supervisor phone number*:+31 317 47 60 24

***** For senior scientist indicate the name of the collaborating colleague

3. Information about the visit

Duration: 3 months

Starting date: 01.09.2008

Ending date: 30.11.2008

4. Description of the activities and outcomes

Background and context: *maximum 10 lines*

The research of the business unit Biointeractions and Plant Health focuses on the analysis and exploitation of plant/pathogen interactions, symbiotic and other microbiological interactions. They develop new strategies and technologies for integrated crop protection, plant health management and ensuring food safety. Team of dr Jan van der Wolff is focused on testing, detection and identification of plant pathogens. identification and use of active organic substances and micro-organisms, genome analyses for the identification of virulent genes of plant pathogens, epidemiological and population dynamic input for decision support systems, and integrated control strategies of pest and diseases.

Objective: *maximum 10 lines*

The project aims to develop technique which is able to distinguish viable from non-viable cells based on the use of propodium monoazide (PMA). PMA only enters bacterial cells when cell membrane is permeablized (such as with dead bacterial cells), where it intercalates DNA. The molecule can be cross-linked by exposure to light. Crosslinked PMA-genomic DNA complexes are removed during standard DNA extraction, whereas the DNA purified from living cells is not affected by PMA presence. This technique has been developed to detect two very important potato pathogens. Gram-positive quarantine phytopathogenic bacterium *Clavibacter michiganensis* subsp. *sepedonicus* (Cms), and Dickeya gram-negative pathogenic bacterium responsible for blackleg in potato.

Activities carried out:

maximum 20 lines

Investigation carried out in PRI laboratory was focused on:

- prep*arat*ion of medium TSA, PCV, YGM.
- optimisation PMA treatment (different concentration of PMA, different exposure time, different concentration of bacteria cells).
- set up of PCR reaction with primers PSA1 PSAR-for *Clavibacter michiganensis* subsp. *sepedonicus*.
- set up of PCR reaction with primers ADE1 ADE2-for *Dickeya*.
- use different kind of antibiotics to estimate the best way of premeablized cell membrane for Diceya.
- genetic transformation of the plant pathogen such as : Xanthomonas spp, Diceya by elactroporation using GFP.
- Staining viable and non-viable cells of *Clavibacter michiganensis* subsp. *sepedonicus*. *Dickeya*. Using SYTO9 in combination with PMA for fluorescence microscopy.
- set up of SEQAM reaction and sequencing for Xanthomonas species.
- set up of Real-Time PCR for Xanthomonas spp.

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5. Links between visit activity and ENDURE

Describe links and relevance of your visit in relation to a specific ENDURE activity(ies) and sub-activity(ies) – maximum 15 lines

My research activities in ENDURE network(seed born diseases) is strictly connected with project which has been realized during my stay in Bionteraction and Plant Health Laboratory in PRI.

The differentiation between live and dead bacterial cells presents an important challenge in many microbiological applications. Due to the persistent of DNA in the environment after cells have lost viability DNA-based detection methods cannot differentiate whether positive signals originate from live or dead bacterial targets. Propodium monoazide (PMA) is highly selective in penetrating only into dead bacterial cells with compromised membrane integrity but not into live cells with intact cell membrane cell walls. Once inside the cells PMA intercalates into the DNA and can be covalently cross linked to it which strongly inhibits PCR amplification. PMA was used to differentiation viable from nonviable cells for two very important potato pathogens Gram positive Cms and Gram negative Diceya, which both are spread by potato seeds. To evaluate seed testing it is necessary to used very sensitive PMA treatment which exclusively detect viable cells allows to avoid “false-positive” results.

6. Impact

Added value for the researcher: *maximum 10 lines*

The main advantage of 3 month scholarship in Wageningen was learning and understanding of use of molecular techniques such as PCR, SEQAM, Real Time PCR genetic transformation of bacterial species. My stay in PRI allows me to develop my knowledge and I had very big opportunity to use my knowledge in practise. Work witch PMA and used it for live and dead distinction have generated very powerful tool for detection of plant pathogen. I have the honour to work with very friendly peoples who help me to understanding many new techniques which I will be able to use in my PhD project.

Added value for sending partner and hosting partner: *maximum 10 lines*

For sending partner: Training PhD student with new technique for detection and identification important bacteria which are causative agents of potato diseases.

For hosting partner: Develop a PMA treatment able to distinguish viable non-viable cells for two important bacterial potato diseases caused blackleg and ring rot in potato tubers. Receiving Xanthomonas species which will be use in microbial ecology research.

Date of submission
2008-12-14

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endure
diversifying crop protection

Dr. Maurizio Sattin
IA3 activity leader

Approved