COST STSM Scientific Report- Jelena Mitrovic

COST Action: FA0807

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STSM Applicant:	**Ms Jelena Mitrovic**, **Institute of Pesticides and Environmental Protection**, **Belgrade(RS)** , jelena.mitrovic@ymail.com
STSM Topic:	**Search for improved methods for phytoplasma strain characterization**
Host:	**Dr. Michael Kube**, **Max Planck Institute for Molecular Genetics**, **Berlin(DE)**, kube@molgen.mpg.de

Background

Strain differentiation is one of the most important tools for performing epidemiological studies aimed at determining the best management practices in order to prevent epidemic outbreaks of phytoplasma diseases. Current classification of phytoplasmas is based on 16S rRNA gene grouping which is a relatively conserved region. However, it is not always suitable for distinguishing strains (subgroups) inside the groups so effectiveness of other genes as markers for characterization of phytoplasma strains is important also.

Purpose of the visit

As well as the general STSM objectives (foster new collaboration, new techniques, use of instruments at the host institute) the goals for this STSM were the complete sequencing of 16S rDNA genes obtained from the phytoplasma collection held at DiSTA (Patologia Vegetale, *Alma Mater Studiorum*, Università di Bologna, Italy) and the identification of new markers for characterization of phytoplasma strains.

Description of the work carried out during the visit

16S rDNA genes from 37 phytoplasma strains maintained in periwinkle (*Catharanthus roseus*) were amplified by using P1/P7 primer pair. For obtaining the purest PCR products (without amplification of adjunctive parts) in order to clone and sequence it afterwards, different PCR conditions such as annealing temperature- 55ºC, 60ºC; annealing time 1min to 30sec; touchdown PCR, different Taq polymerases at different concentrations and $MgCl_2$ at 15mM and 2mM were tested.

The PCR products were then purified and subjected to cloning and sequencing. They were first inserted into plasmid vectors, and then electroporated into competent *Escherichia coli* cells. Recombinant DNA from clones (one clone per strain) was

isolated and completely sequenced by vector based primers as well as 16S specific ones to obtain full sequence coverage.

On the obtained sequences bioinformatical analyses were applied using GAP4 program. First, for each strain sequences were assembled together, and then, after closely looking at the electropherograms, contig sequences were constructed (it included separating vector DNA from phytoplasma DNA).

Candidate genes, useful for diagnostic purposes, were selected by core genome analysis of deduced protein sets of the four completely sequenced phytoplasma genomes using reziclust (http://www.reziclust.molgen.mpg.de).

In addition, DNA was isolated from various phytoplasma infected plant derived materials and amplified using rolling circle amplification (RCA), fragmented by sonification and used for the construction of shotgun libraries.

Analysis of the obtained information is still in progress.