COST Action 0807

Integrated Management of Phytoplasma Epidemics in Different Crop systems

Working group 1

"Planning 2011 activity of WG1 and verifying possibility of screening phytoplasma strain collection by next generation sequencing techniques"

Core group meeting

Belgrade, Serbia, 29th - 30th October 2010

Convenor:
Bojan Duduk

Institute of Pesticides and Environmental Protection
Belgrade-Zemun, Serbia

Program

Friday, 29th October 14:30 – 18:00

Planning 2011 activity of WG1

1. EUPRESCO activity

2. School on bioinformatical analyses of phytoplasma sequences

Saturday, 30th October 9:00 – 16:00

1. Verifying possibility of screening phytoplasma strain collection by next generation sequencing techniques

Minutes of the meeting

Friday, 29th October 14:30 – 18:00

Attendance:
- chair of the COST action: Assunta Bertaccini
- WG1 coordinator: Bojan Duduk
- WG1 member: Michael Kube

Welcome by Bojan Duduk who introduced his Institute and Laboratories

Planning 2011 activity of WG1

The Chair of the Action informed about the need of organize the WG1 activity of 2011 in the best way since this working group is the basis for all Action activity.

1. EUPRESCO activity

After contacts with dr. Ester Torres coordinator of EUPRESCO project on "Interlaboratory comparison and validation of detection methods for phytoplasmas of phytosanitary concern in European orchards" and considering that the harmonization of phytoplasma detection protocols is one of the goals of WG1 we would like to propose a meeting under the COST action for the final preparation of report on results. After consultation with EPPO officer Françoise Petter it was given us the possibility to meet at EPPO headquarters in Paris. The group agrees to present this proposal for MC approval in 2011 budget.

2. School on bioinformatical analyses of phytoplasma sequences

It was proposed to organize a school on the correct sequencing of phytoplasma genes, especially those related to phytoplasma classification and differentiation School. The school should be a joint activity WG1-WG4 following previous suggestion of dr. Hogenhout WG4. Two locations were proposed one in UK and one in Lithuania. Time could be first middle of June 2011, number of students will be defined according to 2011 budget figures but not more than 20 having their own computer.

Discussion about organization

Michael Kube and Bojan Duduk elaborate the following plan:

School should be in June and it should last for 3 days

Michael Kube will write how the Staden package program should be installed in participant computers. The whole day 1. will be spent for work with sequences (5 hours is needed for the lecture about it)

AYWB strain and '*Candidatus* Phytoplasma mali' are good starting point for work with sequences (genes) because AYWB has a lot of repetitive sequences in contrast to the other one that don't have a lot of repetition inside the genome.

Plans will be sent to WG4 leaders for approval and cooperation about lectures (i.e. lectures about the prediction of work of secreted proteins). A lecture about annotation of genes will be given by dr. Kube, and definition of other trainers besides dr. Kube will be organized later after WG1-WG4 leader agreement.

The applicants as trainees should send their CV with the application for the school to WG1-WG4 leaders and after that they will rank applicants and inform which ones will be called to attend the school (suggestion is that 2 trainees should work on one computer), sending to Grant Holder the full list in case replacement will be necessary.

The school could be held in Lithuania, in UK or in another location to be identified by MC.

Saturday, 30th October 9:00 – 16:00

Attendance:
- chair of the COST action: Assunta Bertaccini
- WG1 coordinator: Bojan Duduk
- WG1 member: Michael Kube

Verifying possibility of screening phytoplasma strain collection by next generation sequencing techniques

Dr. Kube presented the subject as summarized below:
Recent advancements in sequencing technology have delivered the so called "Next Generation Sequencing" platforms (NGS), which employ massively parallel approaches to producing millions of sequence reads in a single run (10-100's of millions of short reads, 25-500 bp, in a single run).
This allows to use this platforms for high-throughput resequencing of DNA samples to discover and genotype variants simultaneously.
Various sequencing chemistries and detection technologies are being explored to increase sequencing speed, maximize data output and reduce overall sequencing cost. This way NGS technologies enable the resequencing of entire - genomes or the sampling of entire transcriptomes more efficiently and economically and with greater depth than ever before.
Rather than sequencing individual genomes, now it is possible to sequence hundreds or even thousands of related genomes to sample genetic diversity. Identifying and tracking genetic variation is now so efficient and precise that thousands of variants can be tracked within large populations. To maximize the genomic information generated during a single instrument run these sequencing technologies can be combined with target enrichment.

NGS techniques offer new perspectives also in the analysis of phytoplasmas. One of the major handicaps in phytoplasma genomics is the isolation of sufficient phytoplasma DNA. However, the enormous amounts of sequences resulting from NGS approaches allow to rethink common approaches in phytoplasma genome determination.
Since, so far analyses of conserved genes such as 16srDNA, tuf, Sec did not provide satisfactory strain discrimination, NGS techniques can allow to develop new genetic markers for strain differentiation that could lead to better phytoplasma strain identification as well as can also increase the understanding of host-phytoplasma-vector interaction, epidemiology and on the end lead to a better disease control.

Plans were devised in order to apply the NGS sequences under the supervision of Dr. Kube to a certain number of phytoplasma strains choose among these maintained in the collection of dr. Bertaccini in Bologna; phytoplasma DNA will be isolated from these shoots and also from natural infected *Catharanthus roseus* maintained in potted plants in Belgrade laboratory enriched up to a content of ~5% and sequenced.
Bioinformatical analyses will start with the identification of plant background and phytoplasma DNA to evaluate the potential use of basic enrichment techniques in genome research. Beside the chance to get practice in these cutting edge technologies, *in silico* analysis will also be very relevant part of the work.
The analyses of the anticipated draft sequence(s) will focus on the characterization of the genomic content in comparison to those of completely sequenced phytoplasmas. The anticipated work will lead to the development of new diagnostic markers for some of the phytoplasmas among those more relevant as pathogens in Countries participating to the COST Action.

This collaborative work that can provide important tools for phytoplasma discrimination towards effective disease management will be carried out by tight collaboration between the three laboratory located in Berlin, Belgrade and Bologna respectively but it will be open to all COST participants having some relevant phytoplasma strain that is not enclosed in those present in Bologna-Belgrade laboratories.
It was proposed to further improve this collaboration with a STSM from miss Jelena Mitrovic from Belgrade laboratory considering that she was preliminary trained in a first STSM with basic knowledge about the proposed work.