Final Report STSM:

Viruses associated to *Fusarium circinatum* and endophytes from *Pinus radiata*.

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Purpose of the STSM

The specific objectives of this proposal are:

-To identify putative viral molecules (dsRNA) of the Spanish isolates of *F. circinatum*, particularly possible strains of the genera *Hypovirus* and *Mitovirus*.These viruses are known to decrease the virulence of some other forest pathogens and they could be a sustainble tool for controlling the disease.

-To identify putative viral molecules of the Spanish isolates of endophytes that appear commonly associated to *F. circinatum* in *Pinus radaita* (E.g. *Trichoderm*a spp., *Fusarium* spp.).

-To obtain the sequences of the identified mycoviruses from the *F. circinatum* isolates. The analysis of the mycovirus genome could explain why these viruses affects the pathogen virulence.

Description of the workcarried out during the STSM

A total of 70 samples of 9 differnet species were analyzed for the prcence of Mitoviruses. Two different protocols of Nucleic Acids extraction were carried out. Once the Total RNA had been extracted, PCR was ran for *Fusarium circinatum* isolates with specific primers for Mitoviruses previously found in the Spanish isolates.

1-Samples:

-*Fusarium circinatum* isolated from *Pinus radiata* in Cantabria (Spain). 20 isolates

-*Fusarium circinatum* recieved from FABI (University of Pretoria, South Africa). 29 isolates

- Endophytic fungi isolated from *Pinus radiata* in Cantabria (Spain):

Fusarium oxysporum: 3 isolates

Fusarium sambucinum: 1 isolate

Fusarium avenaceum:7 isolates

Fusarium sporotrichioides: 1 isolate

Fusarium tricinctum:3 isolates

Fusarium beomiforme: 2 isolates

Fusarium cortaderidae: 1 isolate

Trichoderma atroviridae: 3 isolates

2-Samples preparation

Fungal mycelia were culture inPotato Dextrose Agar (PDA) media with a cellophane membrane. Once it had grown it was scraped with a sterilized scalpel. This mycelium was frozen (-20ºC) for 24 h before lyophilization (24h).

3- RNA extractions

Double strained RNA (dsRNA) extraction: DsRNAs molecules were extracted following a modification of the protocol of Morris and Dodds (1979), which is based on the capability of modified CF-11 fibrose cellulose to bind specifically to dsRNA molecules. This extraction was followed by electrophoresis in agarose gel (1%) was run for 1 hour at 120 V. All the samples were analyzed searching for dsRNA.

Total RNA extraction: was run following the protocol described by Vainio et al.,1998. This protocol was used to obtain the cDNA only from *Fusarium circinatum* samples.

Electrophoresis in agarose gel (1%) was run for 1 hour at 120 V.

4-RT reaction

Once, total RNA had been extracted. Reverse Transcriptase reaction was done with the aim of obtain cDNA.

5- Viruses Amplification.

Polymerase Chain Reaction (PCR) with 2 pairs of primers(FCM1F1-FMC1REV1 and FMC3F1-FMC3REV3) was run to check the presence of Mitoviruses in those *Fusarium circinatum* isolates from Cantabria and in those from the International Collection of the University of Pretoria.

Positive and negative controls were used in each run. These primers pairs had been designed from the sequence of those virus species previosly found in *Fusarium circinatum* strains(Unpublished data).

6-Virus sequences

Those PCR products that had been amplified with the above mentioned primers pairs were sent for purification and sequencing to Macrogen (Netherlands).

Obtained partial sequences will be analyzed for knowing the differences among isolates.

Description of the main results obtained

1-RNA Extraction

Double strained RNA (dsRNA) extraction

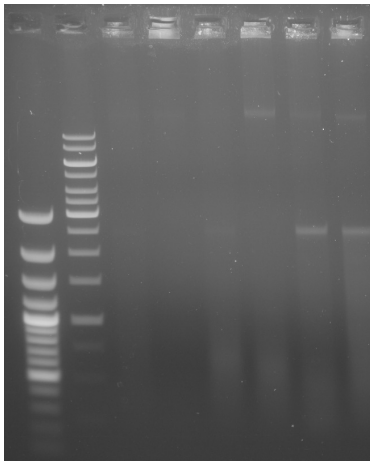

Fig 1: Agarose gel after dsRNA extraction. Wells 7 and 8 showed a clear band at Mitoviruses size.

Double strainded RNA was isolated from 2 samples of *Fusarium circinatum* from Cantabria (Spain).

No dsRNA was found in the analyzed endophytes nor in the *F. circinatum* samples from the international collection.

Total RNA extraction

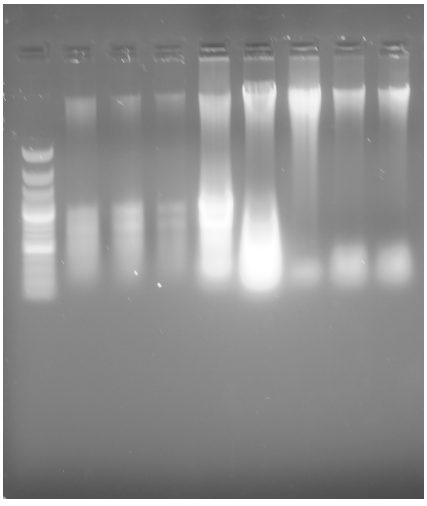

Fig 2. Agarose gel after total RNA extraction.

2-Viruses Amplification

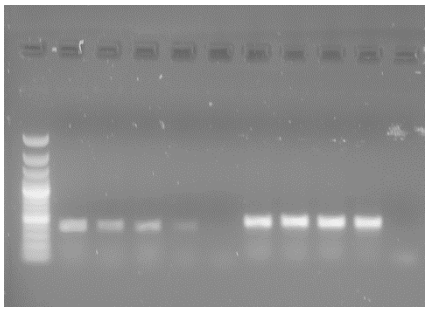

Fig 3. Agarose gel after PCR with primers FMC1(wells from 2 to 6) and FMC3.(wells from 7 to 11).

Five samples of *Fusarium circinatum* collected in Cantabria (Spain) amplified with the primers pairs " FMC1" and "FMC3". All the samples that amplified for one of the primers pair amplified as well for the other one.

The other analyzed samples did not amplify with these primers pairs.

References

Vainio EJ, Korhonen K, Hantula J, 1998. Genetic variation in *Phlebiopsis gigantea* as detected with random amplifies microsatellite (RAMS) markers. Mycol Res 102, 187-192.

Morris, T. J. & Dodds, J. A. (1979). Isolation and analysis of doublestranded RNA from virus-infected plant and fungal tissue. Phytopathology 69, 854–858.

Future collaboration with the host institution (if applicable)

Collaboration METLA-UVa will continue in the frame of the project "Biological control of pitch canker disease with *Fusarium circinatum* mycoviruses"

Foreseen publications/articles resulting from the STSM (if applicable)

After testing the repitability of the different applied methodology and sequences analyses, these data will be published as a collaboration between Finnish Forest Research Institute and University of Valladolid.