Report of the post doctoral activities: Paper abstract and research done during the IDB program

Detection of new plant viruses using the dsRNA extraction strategy

I/Detection of new dsRNA virus infecting *Podosphera* genus

dsRNA extractions was initiated from several fresh leaves of peaches growing in green house. The collected material was showing white filamentous structures of probably powdery mildew. Treated dsRNA was used to cDNA synthesis and cDNA was used as template to amplification with DOP-PCR (degenerated oligonucletide primer PCR). Amplification was only observed with DOP5 primers. The PCR product issued from amplification was purified and ligated in pGEM-Teasy. Ligation product was used to transform XL1 *E.coli* bacteria. Sequencing of cloned fragments and BLASTx and nt revealed the presence of sequences having similarities with viruses' genome. The comparison of protein sequence gives a 76% of homology with RNA polymerase RNA dependant of Partiviridae family. The identified sequence was detected by PCR in fungus infecting peach leaves and in infected leaves but was absent in healthy samples. Investigations confirm that the presence of the identified Partitiviridae virus-like was directly related to the presence of the fungus. Sequencing permits the identification of the fungus which was the specie *Podosphera tridactyla* .

In our analysis, the identified sequence is probably derived from new dsRNA Fungi virus belonging to the Partitiviridea family and the *Partitiviridae* genera.

These investigations will be submitted as a scientific research paper.

II/Metagenomic investigation and new sub-Antarctic viruses infecting *Azorella* genus

In the second part of my work we studied sub-Antarctic plant specie: the symptomatic and asymptomatic *Azorella selago*. Samples were collected from the spontaneous flora of Kerguelen Islands. Lyophilised leaves materials from ten samples were regrouped and used for dsRNA extraction to allow the detection of any virus' presence. Two bands, probably corresponding to dsRNA, were revealed in agarose gel. cDNA fragments were synthesised using random primers and used as template to amplify the whole genome with WGA Kit (GenomePlex Complete Amplification kit, Sigma-Aldrich). PCR products were purified, cloned into pGEM-T Easy vector and sequenced. BLASTx analyses revealed clones with viral sequences presenting an identity with the domain of STV (Southern tomato virus, defining a new taxon related to the *Totiviridae* and *Partitiviridae* families) and the BRVF (Black raspberry virus F unsigned virus). The characterization of the 3'-end of the viral genome was pursued, and confirmed the genomic organization with only one segment dsRNA. Despite these features, the determination of the 5' part of the genome would allow us to precise the genomic organization of this new virus and hence its taxonomical position.

To ensure the origin of this new virus (plant or fungi), samples were tested for the presence of fungi by molecular test using the broad-spectrum fungal primers, ITS-1/ITS-4. These investigations revealed that the presence of the virus is not related to any fungi infecting the *A. selago* samples. In order to precise the prevalence of these virus in *A. selago*, specific primers were designed and used to screen 10 samples. Specific RT-PCR analysis revealed that 4/10 samples were infected with this STV and 3/10 samples for the BRVF.

These preliminary results suggest that new plant viruses infect the genus *Azorella*, a native plant of Kerguelen Islands. These viruses belong to the dsRNA viruses' family.

The dsRNA extraction technique was efficient for diagnosis of new viruses, not detected with classic methods; witch can be the phytpathogene of non characterised diseases. It can be used as complement of the family specific and randomly diagnosis.

This research part will be used for communication in international symposia and can be used to write other research paper.