PhD School on Agriculture, Environment and Bioenergy

(http://sites.unimi.it/dottorato_aab/)

(XXXIX cycle, 2023-26)

Project draft

1.Field of interest

AGR12

2.Project title

3.Tutor (Casati Paola)

- Eventually: co-tutor/s

Passera Alessandro

4.Relevance of the topic and state of the art:

Mycoviruses are found to be infecting fungi. Most of Mycoviruses have a genome with double stranded RNA molecules (dsRNA) and some Mycoviruses genome consist of positive, single stranded RNA (-ssRNA). Moreover, DNA Mycoviruses have been reported recently. These viruses have been detected in almost all fungal phylum but still most of the Mycoviruses remain unknown. Mycoviruses can remain silent or rarely develop symptom in their hosts causing irregular growth, abnormal pigmentation and changes in their host sexual reproduction. For the management of Plant diseases caused by fungi, Mycoviruses can reduce virulence of their host, so they are biocontrol agents. Technically the reduced virulence is called hypovirulence. This hypovirulence phenomena has increased importance of Mycoviruses because it has the potential to reduce the crop losses.
Biological control of mycoviruses is described for *C. parasitica* and CHV: the hypovirulent strains were applied to control the outbreak of the disease in the United States and Europe (Anagnostakis 1982). Other mycoviruses as SsHADV-1 in *Sclerotinia sclerotiorum*, Rosellinia necatrix megabirnavirus 1 (RnMBV1) in *Rosellinia necatrix* (Kondo, Kanematsu, and Suzuki 2013), and *Botrytis cinerea* RNA virus 1 (BcRV1) in *Botrytis cinerea* (L. Yu et al., 2015) were reported induce hypovirulence in the host.

5.Layout of the project (draft)

5.1. Materials & Methods:

Fusarium sp. is an important rice pathogen widely distributed in soil and water. Different isolates will be obtained from rice seed and mono-spore colonies, will be cultivated on PDA medium. dsRNA will be obtained from each fungal colony (Okada et al., 2015). Moreover, the total RNAs of two or three isolates will be extracted extracted using Spectrum plant total RNA kit (Sigma-Aldrich, St. Louis, MO, USA). The dsRNAs and total RNAs extracted of *Fusarium* sp. isolates will be pooled in different samples maintaining an equal proportion of nucleic acid from each isolate. Samples will be then sequenced by Nanopore and Illumina platform. The bioinformatic pipeline adopted is described by Nerva and colleagues (2018). Raw reads will be trimmed, and host sequences removed. Clean reads will be assembled using the metaSPAdes assembler (Prjibelski et al., 2020;). The obtained transcriptome will

be blasted against the viral database using the DIAMOND blastn function and VirFinder. Mapping contigs on the reference viral genomes and ORF predictions will be performed using Geneious Prime software. Specific primers will be designed according to the viral contigs and references viral genome obtained by bioinformatic analysis. Fungal isolates will be tested for the presence of single virus.

5.2. Schedule and major steps (3 years):

1st year
Isolation and characterization of fungal isolates from rice
Total nucleic acid extraction from fungal isolates

I2st year
Metagenomic sequencing
Bioinformatic analysis

3st year
Virome composition of each strain
Evaluating the influence of virome composition on the fungal host

6. Available funds
Risolo

7. Literature:

Anagnostakis, S. L. 1982. “Biological Control of Chestnut Blight.” Science 215 (4532): 466–71.

Kondo, Hideki, Satoko Kanematsu, and Nobuhiro Suzuki. 2013. “Chapter Seven - Viruses of the White Root Rot Fungus, Rosellinia Necatrix.” In Mycoviruses, edited by Said A Ghabrial, 86:177–214. Advances in Virus Research. Academic Press. https://doi.org/https://doi.org/10.1016/B978-0- 12-394315-6.00007-6.

Marzano, Shin-Yi Lee, Berlin D. Nelson, Olutoyosi Ajayi-Oyetunde, Carl A. Bradley, Teresa J. Hughes, Glen L. Hartman, Darin M. Eastburn, and Leslie L. Domier. 2016. “Identification of Diverse Mycoviruses through Metatranscriptomics Characterization of the Viromes of Five Major Fungal Plant Pathogens.” Journal of Virology 90 (15): 6846–63. https://doi.org/10.1128/jvi.00357-16.

Okada, Ryo, Eri Kiyota, Hiromitsu Moriyama, Toshiyuki Fukuhara, and Tomohide Natsuaki. 2015. “A Simple and Rapid Method to Purify Viral DsRNA from Plant and Fungal Tissue.” Journal of General Plant Pathology 81 (2): 103–7.

Prjibelski, Andrey, Dmitry Antipov, Dmitry Meleshko, Alla Lapidus, and Anton Korobeynikov. 2020. “Using SPAdes De Novo Assembler.” Current Protocols in Bioinformatics 70 (1): 1–29. https://doi.org/10.1002/cpbi.102.

Yu, Lin, Wen Sang, Ming De Wu, Jing Zhang, Long Yang, Ying Jun Zhou, Wei Dong Chen, and Guo Qing Li. 2015. “Novel Hypovirulence-Associated RNA Mycovirus in the Plant-Pathogenic Fungus Botrytis Cinerea: Molecular and Biological Characterization.” Applied and Environmental Microbiology 81 (7): 2299–2310. https://doi.org/10.1128/AEM.03992-14.

Nerva, L, G C Varese, and M Turina. 2018. “Different Approaches to Discover Mycovirus Associated to Marine Organisms.” Methods Mol Biol 1746: 97–114. https://doi.org/10.1007/978-1-4939-7683- 6_8.