PhD School on Agriculture, Environment and Bioenergy

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

(XL cycle, 2024-27)

Project draft

1.Field of interest

AGR/12 – Plant pathology

2.Project title

Production of mutants to study the pathogenesis determinants in phytopathogenic bacteria

3.Tutor: Alessandro Passera

co-tutor/s: (to be decided)

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

The interactions between plants and microorganisms have been subject to intense studies in the past decades, due to their strong influence on plant phenotype and productivity. Microorganisms can be both beneficial – commonly defined as plant growth promoting microorganisms (PGPM) – or detrimental to plant growth.

The perspective of current agriculture requires a reduction in the use of synthetic inputs in favor of more sustainable alternatives and, both regarding the optimization of nutrition and the reduction of pesticide use, decoding the plant-microorganism interaction pathways will allow to leverage the great potential that microbiomes hold regarding agricultural production (Schlaeppi and Bulgarelli, 2015; Hirokazu et al., 2018).

Despite this, it is currently not known what differentiates a plant pathogen from a PGPM, as the two categories are not strictly defined on a genetic level and can only be truly differentiated by seeing the outcome of the plant-microbe interaction: different strains of microorganisms within the same species, even with >99% genome homology, might have opposing outcomes when interacting with a same plant host (Sheibani-Tezerji et al., 2015; Passera et al., 2019).

To that end, it is necessary to dissect the genetic and regulatory elements that can affect plant-microorganism interaction to understand which key elements determine the pathogenicity, and subsequently use these elements to achieve a better control of biotic adversities in field.

Among the different kinds of microorganisms, this in-depth study should start from bacteria, as they are the type of microorganism that is most easy to handle in laboratory setting and receptive to genetic manipulation.

5.Layout of the project (draft)

5.1. Materials & Methods:

The project will initially focus on a collection of different strains of the plant pathogenic bacterium *Pseudomonas syringae*, for which a full genome sequence is available as well as a long series of data regarding their phenotype when interacting with plants (Passera et al., 2019). The collection includes different plant pathogenic strains and a plant beneficial strain of *P. syringae*. Using the resources on this collection, the project will identify the main candidate genes that differentiate between the pathogenic and beneficial strains.

Having identified the most promising genes, the sequences will be amplified through PCR and used to produce constructs used to either silence the candidate pathogenicity genes in the pathogenic strains or to express the same genes in the non-pathogenic strain.

Following the transformation of the bacterial strains, the phenotype in plant-bacterial interaction will be tested by inoculating several horticultural crop plants (e.g. tomato, lettuce, cucumber, bean, maize) and evaluating if the knock-out/knock-in of the candidate genes affects the presence or severity of symptoms, as well as the fitness of the mutant in growing in the plant, evaluated both with classical microbiology techniques (plating on selective medium) and through molecular biology (RT-qPCR/RT-dPCR of specific bacterial genes).

After having identified the genes that do actually regulate the pathogenic behavior, the study will be expanded to other bacterial species (e.g. *Xanthomonas campestris*, *Pectobacterium carotovorum*, *Agrobacterium tumefaciens*, *Escherichia coli*) to test whether the knock-in of these genes in a different background than that of *P. syringae* can confer the pathogenicity phenotype, or if the knockout of orthologues of the candidate genes in other plant pathogenic bacteria can reduce or negate the pathogenic phenotype.

Since the candidate genes are very likely to belong to the broad category of regulatory genes (Xie et al., 2019), the mutants will be evaluated both with bioinformatics tools and transcriptomic approaches to determine which genes in the genome of these bacteria react to the presence of the knock-in/knock-out constructs. This last step will allow the identification of genes directly involved with pathogenicity in the molecular interaction with the host, leading to a clarification of the most mechanistic level of plant-pathogen interaction.

5.2. Schedule and major steps (3 years):

The project will follow the following schedule:

1st year:

- Identification of candidate genes;
- cloning of the candidate genes;
- production of the knock-in and knock-out constructs;
- transformation of the *Pseudomonas* isolates with the constructs.

2nd year:

- Assays to describe the phenotypic effects on pathogenicity of the transformation using the produced constructs;
- study of the pathways affected by the transformation in Pseudomonas isolates.

3rd year:

- Transformation of other pathogenic bacteria;
- assay to describe the phenotypic effect on pathogenicity of transformation using the constructs.

6. Available funds (to support research):

PRIN 2022 “MAGICOAT”, 42’400€

7. Co-Financing (to support the bourse):

8. Literature:

Hirokazu et al., 2018, Core microbiomes for sustainable agroecosystems. DOI: 10.1038/s41477-018-0139-4

Passera et al., 2019, Not just a pathogen? Description of a plant-beneficial *Pseudomonas syringae* strain. DOI: 10.3389/fmicb.2019.01409

Schlaeppi and Bulgarelli, 2015, The plant microbiome at work. DOI: 10.1094/MPMI-10-14-0334-FI

Sheibani-Tezerji et al., 2015, The genomes of closely related *Pantoea ananatis* maize seed endophytes having different effects on the host plant differ in secretion system genes and mobile genetic elements. DOI: 10.3389/fmicb.2015.00440

Xie et al., 2019, Regulation of type III secretion system in *Pseudomonas syringae*. DOI: 10.1111/1462-2920.14779