



Master 2 internship proposal

2027

TITLE: Role of fungal peptides (RiPPs) produced by plant pathogenic fungi

Supervisors : Muriel Viaud et Laure Bardiot

Laboratory/Department/Institute: INRAE-BIOGER, Equipe Effecteurs de la Communication Cellulaire à l'interface Champignon-Plante (ECCP)

Address, e-mail: 22 place de l'Agronomie, 91120 Palaiseau

To apply, please send your resume and a cover letter to muriel.viaud@inrae.fr and laure.bardiot@inrae.fr

INTRODUCTION, SCIENTIFIC CONTEXT:

Fungi produce a wide range of bioactive secondary metabolites (SMs) acting as chemical effectors to colonize their ecological niche. In particular, phytopathogenic species produce SMs that act as phytotoxins (Dalmais et al., 2011) or effectors manipulating the physiology of their host (Collemare et al., 2019) but also SMs to compete with other microorganisms (Vignatti et al., 2020). Among fungal SMs, a new family of peptides called RiPPs (for ribosomally synthesized and post-translationally modified peptides) recently attracted attention as they could act as phytotoxins (Ustiloxins, Phomopsins) or effectors manipulating plant immunity (Victorin) (Ford et al., 2022).

RESEARCH PROPOSAL:

The global aim of the project is to understand the role of RiPPs in *Botrytis cinerea*, a polyphagous fungus that infects grapevine, tomato and hundreds of other host plants and interact with numerous microorganisms present in the phyllosphere (other filamentous fungi, yeasts, bacteria). Genome mining and transcriptomic studies revealed that *B. cinerea* carries 9 RiPP biosynthetic gene clusters (BGCs) and that some of them are upregulated during plant infection



suggesting a role as effectors of pathogeny (L. Bardiot'PhD; ANR project FRiPPon).

The main objective of the Master's 2 program would be to conduct a functional analysis of one of these candidate BGCs to identify the chemical structure of the RiPP and its role in plant infection. To this end, the student will express the biosynthetic genes of this BGC in a heterologous host (*Escherichia coli*), purify the enzymes and synthesize the RiPP *in vitro*, an approach described by Chiang et al. (2025). The structure of the produced RiPP will then be characterized at the Museum National d'Histoire Naturelle (Paris). Finally, bioassays will be initiated to evaluate the bioactivities of the produced RiPP on host plants.

In parallel, the student will explore new publicly available RNAseq data to determine whether some of the 9 RiPP BGCs of *B. cinerea* are upregulated during interactions with other fungi (*Trichoderma atroviride*, *Hyphopichia pseudoburtonii*) thus suggesting potential antifungal activities. This will be done thanks to the support of Adeline Simon (bioinformatic platform of Bioger). Expression patterns could be confirmed and refined by co-cultivating *B. cinerea* fluorescent reporter lines already available in the lab (promoter of RiPP biosynthetic gene + GFP or mCherry fluorescent protein) with a broader set of microorganisms, including those that co-occur with *B. cinerea* on the phyllosphere (Vignatti et al., 2020).

Overall, this project will contribute to the discovery of new bioactive natural products that are involved in biotic interactions and possibly to find applications in agriculture as well as in the field of health.

METHODOLOGIES:

- Microbiology: cultivation of fungi and bacteria, co-cultivations.
- Molecular biology: Transformation of *E. coli* with heterologous expression vectors (already designed and synthesized by Genescript), DNA extraction and PCR validation, protein extraction
- Biochemistry: Western-blot, Protein purification, *in vitro* synthesis.
- Chemistry: extraction, HPLC (MS in collaboration with natural product chemists at the Museum National d'Histoire Naturelle, Paris)



- Bioassays on plants (*Arabidopsis*, tomato)
- Epifluorescence microscopy

REFERENCES (maximum 5):

- Chiang CY, Ohashi M, Tang Y. Copper-Dependent Oxidase Catalyzes Aryl Cross-Coupling in the Biosynthesis of a Minimal Ribosomally Derived Natural Product. *J Am Chem Soc.* 2025 Dec 31;147(52):48311-48319. doi: 10.1021/jacs.5c17946. Epub 2025 Dec 15. PMID: 41398156.
- Collemare J, O'Connell R, Lebrun MH. Nonproteinaceous effectors: the terra incognita of plant-fungal interactions. *New Phytol.* 2019 Jul;223(2):590-596. doi: 10.1111/nph.15785. Epub 2019 Apr 3. PMID: 30851201.
- Dalmais B, Schumacher J, Moraga J, LE Pêcheur P, Tudzynski B, Collado IG, Viaud M. The Botrytis cinerea phytotoxin botcinic acid requires two polyketide synthases for production and has a redundant role in virulence with botrydial. *Mol Plant Pathol.* 2011 Aug;12(6):564-79. doi: 10.1111/j.1364-3703.2010.00692.x. Epub 2011 Jan 17. PMID: 21722295; PMCID: PMC6640383.
- Ford RE, Foster GD, Bailey AM. Exploring fungal RiPPs from the perspective of chemical ecology. *Fungal Biol Biotechnol.* 2022 Jun 25;9(1):12. doi: 10.1186/s40694-022-00144-9. PMID: 35752794; PMCID: PMC9233826.
- Vignatti P, Gonzalez ME, Jofré EC, Bolívar-Anillo HJ, Moraga J, Viaud M, Collado IG, Pieckenstein FL. Botrydial confers Botrytis cinerea the ability to antagonize soil and phyllospheric bacteria. *Fungal Biol.* 2020 Jan;124(1):54-64. doi: 10.1016/j.funbio.2019.11.003. Epub 2019 Nov 15. PMID: 31892377.