Proposition de stage

Parcours Master 2 « Microbiologie, Environnement, Santé »

1. **Laboratoire / Entreprise d'accueil :**

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2. **Titre, description du sujet, approches utilisées, références** (1 page maximum) **:**

TITLE: Pathogen lifestyle in non-host plants
CONTEXT: The pathogen *Agrobacterium tumefaciens* provokes irreversible damage on plants via genetic modification of the plant host leading to tumor formation. The mechanisms underlying *Agrobacterium* virulence are well known: under favorable conditions (plant wounding), the pathogen transfers a part (the T-DNA) of its virulence plasmid (the Ti plasmid) into the genome of infected plant cells. The genetically modified host cells are reprogrammed to proliferate and to form tumors that are colonized by *A. tumefaciens*.
Almost 400 plant species are listed as hosts for *Agrobacterium*, though strains of this genus may exhibit different aggressiveness towards one or the other hosts. Millions of dollars of economic losses are associated with the Agrobacterium-induced crown gall disease every year. In addition to prophylactic actions, some disease control procedures exist but they remain limited to a few pathogenic strains. More effective treatments against *A. tumefaciens* are therefore needed. Crown-gall disease management may expect progress, thanks to an increased knowledge of plant defense mechanisms and further - but still quite rare - studies on pathogen traits involved in plant colonization.
Apart from the constructed ecological niche of the tumors on host plants, *Agrobacterium* populations are common although not abundant in the root microbiota of hosts and non-hosts. Hence, *Agrobacterium* populations are able to colonize asymptomatically roots of unwounded plant hosts in which they may reside without initiating a virulence process. Yet, a global, comparative picture of the fitness genes that contribute to the different lifestyles of *Agrobacterium* in symptomatic and asymptomatic plants is not known. This knowledge could help us to develop chemical and biological treatments to limit colonization of plants by *A. tumefaciens* and so to prevent settlement and infection processes.

RESEARCH PROPOSAL : In a recent work (Torres et al submitted), we used transposon-sequencing (Tn-Seq) to investigate the fitness genes of *A. tumefaciens* C58 involved in the colonization of the tomato host plant (roots and tumors). In this M2-project, we are proposing to identify the *A. tumefaciens* fitness genes that are associated to colonization of the non-host plant maize. This work is an important step to understand how *A. tumefaciens* could survive in non-host plant reservoirs. This work is organized in two task: (1) plant inoculations by a Tn-mutant population of *A. tumefaciens* C58 to identify fitness genes by Illumina sequencing; (2) validation of the candidate genes by reverse genetic using *in planta* competitions between the *A. tumefaciens* C58 wild type and constructed mutants.

METHODOLOGIES :
- Task1: the library of Tn-mutants is already constructed (Gonzalez-Mula et al., 2019), so infection assays will be performed in greenhouse at I2BC and then Tn-mutants will be recovered from maize roots and total DNA will be extracted to perform Illumina sequencing at the I2BC platform.

- Task2: because we already obtained a set of data from task1 in a previous experiment, a couple of candidate fitness genes will be chosen and KO-mutants will constructed by reverse genetics. Then, competitions between WT and mutant strains will be performed in the greenhouse.
This project combined molecular microbiology and plant-host interaction approaches.

REFERENCES (maximum 5) about the team on the topic

- A. Gonzalez-Mula, J. Lachat, L. Mathias, D. Naquin, F. Lamouche, P. Mergaert, D. Faure. 2019. The biotroph Agrobacterium tumefaciens thrives in tumors by exploiting a wide spectrum of plant host metabolites. New Phytologist 222: 455–467.

González-Mula A., J. Lang, C. Grandclément, D. Naquin, M. Ahmar, L. Soulère, Y. Queneau, Y. Dessaux, D. Faure. (2018). Lifestyle of the biotroph Agrobacterium tumefaciens in the ecological niche constructed on plant host. New Phytologist 219: 350-362.

- Tannières, M., Lang, J., Barnier, C., Shykoff, J.A., and Faure, D. (2017). Quorum-quenching limits quorum-sensing exploitation by signal-negative invaders. Scientific Reports 7, 40126.

- Lang J., Vigouroux A., El Sahili A., Kwasiborski A., Aumont-Nicaise M., Dessaux Y., Shykoff J.A., Moréra S., and Faure D. (2017). Fitness costs restrict niche expansion by generalist niche-constructing pathogens. ISME J 11, 374-385. doi : 10.1038/ismej.2016.137.

- Lang, J., Gonzalez-Mula, A., Taconnat, L., Clement, G., and Faure, D. (2016). The plant GABA signaling downregulates horizontal transfer of the Agrobacterium tumefaciens virulence plasmid. New Phytologist 210, 974-983. doi : 10.1111/nph.13813.