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Project application form

- **Research Unit of the PhD student:** Laboratory of Plant-Microbe Interactions (LIPM)

- **Thesis supervisor:** Stéphane GENIN (HDR)
- **Co-Supervisor:** Alice GUIDOT

- **Number of post-docs and PhDs currently supervised :** 1

- **Thesis project title:** Role of epigenetic modifications in a plant pathogen during host adaptation

- **Thesis project description (1.5 page maximum) (Scientific context, Previous results, Project description, References)**

Over the last decade, it has become clear that some of the bacterial cells in an isogenic population behave differently from the others. This heterogeneity provides a sort of bet hedging strategy, ensuring that there will always be some cells with the right phenotype, or right evolvability, for the changing environment[1]. Phenotypic switching, either caused by phase variation or by epigenetic mechanisms, has been mostly described in animal pathogens[2]. Microbial pathogens indeed constantly alternate between their host and the compartment they disperse in.

Epigenetic modifications are heritable changes in gene expression that occur without changes in the DNA sequence. They are responsible of important phenotypic trait variations in all kingdoms of life and are often caused by external factors, typically environmental changes. DNA methylation is the most common form of epigenetic modification in prokaryotic and eukaryotic genomes and is catalyzed by DNA methyltransferase (MTase) enzymes[3]. To date, detection of methylated DNA has posed significant technical problems. Recently, the development of the PacBio sequencing technology has enabled sequencing of single molecules in real time (SMRT) without amplification. Interestingly, this new sequencing technology has the potential to also detect methylated bases (e.g. 6mA, 4mC, 5mC)[4].

In bacteria, the biological consequences of DNA methylation have not been extensively investigated yet. The impact of DNA methylation in pathogenicity has been reported in some animal pathogenic bacteria[5]. However, the involvement of DNA methylation in host-pathogen interaction remains largely unexplored for most of the bacterial plant pathogens. The main purpose of this project is to study a plant pathogenic bacterium, *Ralstonia solanacearum*, to describe the DNA methylation patterns and to focus on the involvement of DNA methylation during host adaptation.

R. solanacearum is the causal agent of the plant bacterial wilt disease, recognized as one of the most destructive plant bacterial diseases. *R. solanacearum* is an attractive model for studying the mechanisms governing host adaptation since this bacterium has an unusual wide host-range of more than 250 plant species[6]. In order to investigate the genetic bases of host adaptation in *R. solanacearum,* we conducted an experimental evolution project with the GMI1000 strain by serial passage experiments of a single clone on eight different host plants over 300 bacterial generations. We demonstrated that almost all experimentally evolved clones had a fitness gain in their experimental host compared to the ancestral clone[7]. However, only few (even no) DNA polymorphisms could be detected in their genomic sequence. In this thesis project, we aim to assess the hypothesis that, in addition to DNA sequence

modifications, the *in planta* fitness increase detected in the evolved clones could also be the results of epigenetic modifications.

The project will be organized in four parts:

(1) The first part will address the MTases in the *R. solanacearum* GMI1000 strain. In a pilot experiment, the GMI1000 genome has been sequenced using the SMRT technology. This analysis already revealed two major motifs in the GMI1000 genome that are essentially always methylated. The role of each GMI1000 MTase in virulence will be investigated by generating single-deleted mutants using protocols that are routinely used in our group. The impact of MTase single-deletion on the GMI1000 methylome profile will be determined using SMRT sequencing technology. For the analysis of the SMRT sequence data, existing bioinformatic tools, that are freely made available by PacBio, have been adapted by the LIPM bioinformatic team for the high GC content genomes, such as the *R. solanacearum* genome.

(2) The second part will address the MTase repertoires and the methylome profiles in 20 wild-type strains representative of the *R. solanacearum* genetic diversity. This should provide a global overview of the methylome pattern diversification in *R. solanacearum* and potential correlation with host specificity.

(3) The third part will address the methylome diversification in 30 experimentally evolved clones, derived from the GMI1000 strain after serial passages during 300 generations in a given host. The methylome of the ancestral GMI1000 strain and the 30 evolved clones will be compared in order to identify differential methylation marks. The transcriptome profiles of these evolved clones will also be investigated in order to connect differential methylation marks in gene promoters with transcriptome profile variation.

(4) The last part of the project will focus on interesting candidate methylation marks varying between the experimentally evolved clones and the ancestor and found in several independent evolved clones (parallel variations). This will provide insights into the overall reproducibility of evolutionary changes. For the most interesting candidates, the effect of individual methylation marks on the *in planta* fitness will be characterized. For that purpose, we will conduct site-directed mutagenesis in the ancestral GMI1000 clone and then measure the fitness of the mutants *in planta* using protocols previously described[8]. This should provide insights into the role of some methylation marks in GMI1000 adaptation to different host plant species.

References

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3. **Clark, T. A. *et al.* Characterization of DNA methyltransferase specificities using single-molecule, real-time DNA sequencing. *Nucleic Acids Res.* 40, e29–e29 (2012).**
4. **Schadt, E. E. *et al.* Modeling kinetic rate variation in third generation DNA sequencing data to detect putative modifications to DNA bases. *Genome Res.* 23, 129–141 (2013).**
5. **Casadesús, J. & Low, D. Epigenetic Gene Regulation in the Bacterial World. *Microbiol. Mol. Biol. Rev.* 70, 830–856 (2006).**

6. Peeters, N., Guidot, A., Vailleau, F. & Valls, M. *Ralstonia solanacearum*, a widespread bacterial plant pathogen in the post-genomic era. *Mol. Plant Pathol.* 14, 651–662 (2013).
7. Guidot, A. *et al.* Multihost Experimental Evolution of the Pathogen *Ralstonia solanacearum* Unveils Genes Involved in Adaptation to Plants. *Mol. Biol. Evol.* 31, 2913–2928 (2014).
8. Macho, A. P., Guidot, A., Barberis, P., Beuzón, C. R. & Genin, S. A competitive index assay identifies several *Ralstonia solanacearum* type III effector mutant strains with reduced fitness in host plants. *Mol. Plant-Microbe Interact.* 23, 1197–1205 (2010).

- **Project summary (200 words) :**

In an isogenic population, bacterial heterogeneity is a sort of strategy ensuring that some cells have the right phenotype for changing environments. DNA methylation is an underlying epigenetic mechanism that causes bacterial heterogeneity. It is catalyzed by DNA methyltransferase (MTase) enzymes. In bacterial pathogens, the role of DNA methylation has been reported in animal pathogens but remains largely unexplored for plant pathogens. The objective of this project is to investigate the role of DNA methylation during host adaptation in the plant pathogen *Ralstonia solanacearum*. For that purpose, the MTases in the GMI1000 strain, their role in virulence and their impact on the GMI1000 methylome pattern will be investigated. The methylome patterns will be determined using the PacBio sequencing technology. In order to have a global overview of the methylome pattern diversification in *R. solanacearum*, the MTase repertoires and the methylome patterns in 20 wild-type strains will be compared. The methylome diversification in 30 experimentally evolved clones, derived from GMI1000 after serial passages during 300 generations in a given host, will then be addressed. In order to investigate the role of some methylation marks during adaptation to different hosts, the effect of individual methylation marks on the *in planta* fitness will be characterized.