# Project 4

## Characterization of the mechanism of action of the NAP Lrp in the regulation of chromosome architecture and virulence gene expression in *Dickeya dadantii*.

**Background.**

Bacterial pathogens face stress from host defenses and must coordinate virulence factor production to optimize energy use, ultimately leading to bacterial dispersal. This adaptation requires genetic reprogramming, mainly at the level of transcriptional initiation. Nucleoid-Associated Proteins (NAPs) regulate chromatin structure locally by folding, bridging, and wrapping DNA, while maintaining global DNA supercoiling through topoisomerase interactions. These functions exert long-range regulatory effects on transcription, positioning NAPs as global gene expression regulators that control bacterial fitness in host environments. **Most NAP studies have focused on animal pathogens like *E. coli, Salmonella,* and *Shigella*, but limited research has explored their role in phytopathogenic bacteria. Further results on bacteria living in different ecological niches may provide a better understanding of how NAPs and chromosome dynamics co-ordinate the establishment of acclimation processes and the expression of bacterial virulence.** *Dickeya* are plant-pathogenic Gram-negative bacteria causing soft rot and significant agricultural losses. Pectinolytic *Dickeya* species have gained attention due to their recent global emergence and impact on important crops like potato and chicory. *Dickeya dadantii* is an ideal model for this study due to its extensive characterization and available molecular tools. The virulence of these necrotrophic bacteria is linked to their secretion of plant cell wall-degrading enzymes, including pectinases, as well as factors like flagella and adhesins that facilitate bacterial adhesion. *D. dadantii* also possesses adaptive systems to thrive in the plant's hostile intercellular spaces, such as nutrient scarcity and acidic pH. In *E. coli* and *Salmonella enterica*, the NAP Lrp (Leucine Responsive Regulatory Protein) regulates up to 10% of the bacterial genome, with its activity influenced by leucine, particularly in amino acid metabolism. Lrp is also involved in virulence regulation in animal pathogens like *S. enterica*, *E. coli*, and *Vibrio cholerae*. Preliminary results obtained by the team show that a *D. dadantii* mutant lacking Lrp exhibits reduced virulence and decreased production of early virulence factors. Transcriptomic and ChIP-seq analyses under different *in vitro* conditions suggest that Lrp acts also as a global transcriptional regulator of virulence genes in *D. dadantii*.

**Objectives.**

**The aim of this PhD project is to investigate how NAPs coordinate chromosomal architecture and regulate the transcription of virulence and adaptive genes in the model bacterium *Dickeya dadantii* with a particular focus on elucidating the mechanism of action of the NAP Lrp (Leucine Responsive Regulatory Protein). We hypothesize that the Lrp plays a key role in the acclimatization of *D. dadantii* to the hostile host conditions in particularly under nutritional deficiency. Our goal is to unravel the mechanisms by which *D. dadantii* utilizes Lrp to establish itself during the early phases of infection and ultimately trigger the symptomatic phase.**

**Scientific program and Methodology.**

**Contribution of Lrp to the chromosomal architecture of *D. dadantii*:**

Generation and comparison of chromosomal contact maps (Hi-C data) of the *lrp* mutant with those of the wild-type strain already available in the team under various conditions (standard, relevant stress conditions and during plant infection). These data will be correlated with ChIP-Seq and RNA-seq data obtained by the team for the wild-type and *lrp* mutant strains in order to link chromosome conformations to activities of specific genes affected during the pathogenic growth of *Dickeya*.

**Contribution of Lrp to DNA supercoiling:**

Negative supercoiling in the *lrp* mutant will be analyzed using the Psora-seq technique, while positive supercoiling will be mapped with the GapR-Seq method, recently optimized by our team for the wild-type *D. dadantii* strain.

**Structural analysis of Lrp-DNA complexes:**

The interaction Lrp-DNA, already identified through ChIP-seq, RNA-seq, and EMSA experiments, will be further investigated using the team's expertise in DNAse I footprinting. To achieve deeper structural insights, complementary biophysical techniques—including crystallography, NMR spectroscopy, atomic force microscopy, and Dynamic Light Scattering—will be employed in collaboration with the facilities at the ISBG in Grenoble.

**Functional analysis of Lrp regulation:**

The investigation of how Lrp interacts with key virulence gene regulators and integrates environmental signals (e.g., leucine) to modulate DNA architecture and gene expression will be conducted using the team's expertise. This will involve genetic analyses (knockout mutants), protein-protein interaction assays, and dynamic localization studies using 3D SMLM imaging and single-particle tracking, in collaboration with Joanna Timmins at the IBS in Grenoble.

**This PhD project will use interdisciplinary approaches, including biochemistry, genetics, and biophysics, to investigate the role of the NAP Lrp.** The findings will provide insights into Lrp's distribution, its interactions with regulators of virulence gene expression, and its impact on nucleoid organization in *D. dadantii* during plant infection. The research will enhance our understanding of how *D. dadantii* coordinates virulence gene expression and drives infection, potentially paving the way for novel strategies to disrupt bacterial infections and develop innovative treatments for plant health.

Supervisor(s) name(s), Affiliation(s), eMail address(es) for contact:
**Cécile RIBOT, UCBL, cecile.ribot@univ-lyon1.fr**
**Sam MEYER, INSA Lyon, sam.meyer@insa-lyon.fr**

Proposed collaboration within ArchiFun network (not mandatory at this stage):

Proposed list of secondments (not mandatory, but recommended if known already):

Main ArchiFun theme involved:

- Host-pathogen interactions;
- Mechanisms of bacterial resistance and cancer onsets;
- Neurodegenerative and autoimmune diseases;
- Translational research in prevalent diseases;
- Physiology and ecology;
- Neurosciences and cognition.