**M2 Internship proposal 2023 - 2024**

**Team**

Team: Plant-Bacteria Interactions

Team website: https://www.i2bc.paris-saclay.fr/equipe-plant-bacteria-interactions/

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**Title of the M2 project**

**The role of the outer membrane of *Caballeronia insecticola* in the resistance to antimicrobial peptides and the colonization of plant roots and insect guts**

**Project description**

**Project:**

The bacterium *Caballeronia insecticola* has multiple lifestyles: 1) it is a gut resident of the phytophagous insect *Riptortus pedestris*; 2) it is a soil bacterium that is well adapted to survive in this oligotrophic environment; 3) in soils, it can associate with hyphae of fungi and travel over long distances using growing hyphae as vehicle; 4) it can colonize the rhizosphere and endosphere of plants.

High-throughput genetic screens in *C. insecticola* have been performed in the recent past by the laboratory, using transposon-sequencing (Tn-seq) in order to identify the genetic repertoire that determines its adaptation to these environments. In addition, similar genetic screens have been performed on in vitro conditions that might mimic particular stress or growth conditions existing in the natural environments.

In a Tn-seq screen with membrane-targeting antimicrobial peptides (AMPs), which are abundantly produced in the insect gut, many genes in lipopolysaccharide (LPS) biosynthesis were identified in addition to genes with unknown function. Interestingly, these genes were also identified in the screens performed in the insect gut as well as in the rhizosphere colonization, indicating that AMP-resistance is crucial to colonize the insect gut and the plant rhizosphere.

Contrary to the often-held view that LPS is the primary AMP-target in the outer membrane of bacteria, our detailed molecular characterization of the various *C. insecticola* AMP-resistance mutants strongly suggests that in *C. insecticola* this is not the case. Our data rather suggest that the LPS form a physico-chemical shield that prevents the AMPs to reach their real but so far unknown targets in the outer membrane of the bacteria, which could be other outer membrane lipids or proteins. The identification in our initial AMP Tn-seq screen of gene functions unrelated to LPS biosynthesis is in agreement with this hypothesis.

In order to go towards the better understanding of how AMPs interact with *C. insecticola* membranes and the identification of their molecular targets, the Master 2 student will perform new genetic screens. The available AMP-sensitive mutants (LPS mutants and mutants in other functions) will be mutagenized by transposon mutagenesis. These libraries will be screened for mutations that revert the AMP-sensitivity to wild-type level resistance. Such mutants could have the AMP-targets inactivated. The screens will be performed by Tn-seq as well as by selection of resistance clones. In parallel, flow cytometry combined with Tn-seq will be used to identify mutants that have an altered binding to fluorescent-labeled AMPs. Such mutants are also expected to be in genes involved in the constitution of the AMP membrane targets.

Deletion mutants of thus-identified candidate genes will be constructed by the student. Finally, the mutants will be phenotypically characterized in detail in terms of AMP resistance and capacity to colonize the natural environments of the bacterium.

**Methods used during the internship:**

-Tn-seq: the creation of transposon mutant libraries, AMP-sensitivity and flow cytometry-based screenings and high throughput sequencing for quantification of all mutants in the mutant population.

-Molecular microbiology for construction of deletion mutants.

-Mutant phenotyping assays: AMP-sensitivity assays; fluorescence microscopy and flow cytometry; chemical analysis of LPS; other chemical and biological analytic methods depending on the nature of the mutated genes.

-Insect rearing, infection, dissection and gut analysis by microscopy and flow cytometry.

-Plant growth and colonization and analysis by bacterial counting.

**Keywords**

**Antimicrobial Peptides; Tn-seq (transposon sequencing); bacterial outermembrane and resistance; *Caballeronia insecticola* (belonging to the *Burkholderiaceae*)**

**Bibliography**

van Opijnen, T., and Levin, H.L. (2020). Transposon insertion sequencing, a global measure of gene function. Annu. Rev. Genet. 54: 337-365. Doi: [10.1146/annurev-genet-112618-043838](https://doi.org/10.1146/annurev-genet-112618-043838)

Mergaert, P. (2018). Role of antimicrobial peptides in controlling symbiotic bacterial populations. *Nat. Prod. Rep.* 35, 336-356. doi: 10.1039/c7np00056a.

Mergaert, P., Kikuchi, Y., Shigenobu, S., and Nowack, E.C.M. (2017). Metabolic integration of bacterial endosymbionts through antimicrobial peptides. *Trends Microbiol.* 25, 703-712. doi: 10.1016/j.tim.2017.04.007.

**Other selected publications of the team**

Travin, D.Y., Jouan, R., Vigouroux, A., Inaba-Inoue, S., Lachat, J., Haq, F., Timchenko, T., Sutormin, D., Dubiley, S., Beis, K., Moréra, S., Severinov, K., and Mergaert, P. (2023). A dual uptake mode of the antibiotic phazolicin by Gram-negative bacteria prevents resistance acquisition. *mBio* 14, e00217-23. doi: 10.1128/mbio.00217-23.

diCenzo, G., Cangioli, L., Nicoud, Q., Cheng, J.H.T., Blow, M.J., Shapiro, N., Woyke, T., Biondi, E.G., Alunni, B., Mengoni, A., and Mergaert, P. (2022). DNA methylation patterns in bacteria of the genus *Ensifer* during free-living growth and during nitrogen-fixing symbiosis with *Medicago* spp. *mSystems* 7, e01092-21. doi: 10.1128/mSystems.01092-21.

Nicoud, Q., Barrière, Q., Busset, N., Dendene, S., Travin, D., Bourge, M., Le Bars, R., Boulogne, C., Lecroël, M., Jenei, S., Kereszt, A., Kondorosi, E., Biondi, E.G., Timtchenko, T., Alunni, B., and Mergaert, P. (2021). *Sinorhizobium meliloti* functions required for resistance to the antimicrobial NCR peptides and bacteroid differentiation. *mBio* 12, e00895-21. doi: 10.1128/mBio.00895-21.

Jang, S., Mergaert\*, P., Ohbayashi, T., Ishigami, K., Shigenobu, S., Itoh, H., and Kikuchi, Y. (2021). Dual oxidase enables insect gut symbiosis by mediating respiratory network formation. *Proc. Natl. Acad. Sci. USA* 118, e2020922118. doi: 10.1073/pnas.2020922118.

Van de Velde, W., Zehirov, G., Szatmari, A., Debreczeny, M., Ishihara, H., Kevei, Z., Farkas, A., Mikulass, K., Nagy, A., Tiricz, H., Satiat-Jeunemaître, B., Alunni, B., Bourge, M., Kucho, K., Abe, M., Kereszt, A., Maorti, G., Uchiumi, T., Kondorosi, E., and Mergaert, P. (2010). Nodule specific peptides govern terminal differentiation of bacteria in symbiosis. Science 327, 1122-1126.

**Perspectives**

The M2 internship can be followed by a PhD thesis

Doctoral School of reattachment: ED 567, Sciences du végétal : du gène à l'écosystème (SEVE)