Proposition de stage

Parcours Master 2 « Microbiologie, Environnement, Santé »

1. Laboratoire / Entreprise d'accueil :

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2. Description du stage (2 pages maximum) :

Titre : Chemotaxis in the Burkholderia insecticola – bean bug symbiosis

Mots clés : chemotaxis; bacteria-insect symbiosis

Contexte et objectifs généraux :
Many stinkbugs are notorious pests of crops, including among the most important crops on a global level (e.g. rice, soybean). For normal development and growth, optimal reproduction and in certain cases survival, stinkbugs require symbiotic bacteria. These symbionts are maintained in a specific region of the midgut, the M4 region, composed of crypts that harbor the bacterial symbionts. The bean bug *Riptortus pedestris* is the model species for the study of this symbiosis. Its natural hosts are the legumes soybean, pigeon pea, cowpea and chickpea on which it provokes serious crop losses by damaging seeds, rendering them unusable for consumption. Newborn bean bugs are symbiont free and the symbionts are acquired from the soil or the plant environment by every new generation of the insect at the second instar. The insects can be maintained in the laboratory apo-symbiotically (symbiont free) and infected with a desired strain by inoculating the strain in drinking water. In nature, *R. pedestris* has a very high specificity for symbiont species like *Burkholderia insecticola* and related species of the *Burkholderia*, while more distantly-related bacteria, are entirely unable to colonize the midgut crypts.

Projet de stage :
Recently, we made a transposon sequencing (Tn-seq) analysis of *B. insecticola* that provided a complete picture of the 'fitness landscape' of this bacterium in the symbiotic organ of the insect. Tn-seq is a high-througput genetic screen that characterizes transposon-mutant bacterial populations by Illumina sequencing and that thereby identifies genes required for bacterial fitness in a condition of interest. This data revealed, among many other functions, that motility and chemotaxis are key functions of *B. insecticola* to colonize the midgut crypts. All mutants in genes required for the construction of the flagellar machinery and for chemotactic signaling were unable to colonize the midgut. We even identified the specific chemoreceptors (two receptors out of 13 in the genome) that are required for the colonization. This indicates that the midgut crypts produce specific chemoattractants that guide the symbiotic bacteria towards the symbiotic organ during its infection. We propose in this Master 2 project to characterize this chemotaxis in detail.

APPROACHES AND METHODOLOGIES
1) The panel of chemical compounds that can act as chemoattractant for *B. insecticola* will be determined. For this, a large collection of compounds will be screened in a fast assay based on visual inspection of bacterial motility towards the compounds of interest on soft agar plates. The compounds that will be tested will include sugars, amino acids, other nitrogen compounds, inorganic ions and compounds that are potentially produced by the insect (based on our RNA-seq and Tn-seq data sets).

Chemotaxis towards positive compounds will then be further characterized more quantitatively using a capillary assay and flow cytometry based quantification of bacterial movement.
2) In parallel, a metabolomic analysis of the midgut crypts in aposymbiotic insects will determine the relative abundance of metabolites in this organ. This list will be matched with the chemoattractant compounds identified in 1). This will involve insect rearing and dissection of insects to harvest the symbiotic organ. The metabolome analysis will be performed by a specialized platform.
3) *B. insecticola* mutants will be created in the two chemoreceptors that were found by Tn-seq to be essential for crypt colonization. Mutants will be created by standard molecular microbiology methods. The chemotactic response of these mutants towards the panel of compounds identified in 1) and 2) will be determined to identify the compounds that are recognized via these chemoreceptors.
4) The symbiotic phenotype (colonization of the insect midgut crypts) of the mutants in the chemoreceptors made in 3), as well as available mutants in the chemotaxis-signaling cascade will be analyzed in detail. This will involve insect rearing and infection with the mutant strains expressing fluorescent proteins (GFP, RFP, YFP, or CFP), dissection of insects to harvest the symbiotic organ and microscopy and flow cytometry analysis of the gut content to determine the infection rate. Furthermore, we will explore the possibility to prevent infection by the use of chemical compounds that can interfere with the chemotaxis.

Les objectifs de ce stage M2 sont :

The project will discover the chemoattractants produced by the insect. Interference with this chemotaxis process could be developed to prevent the establishment of the symbiosis as a novel strategy in pest control.
The student will be trained in molecular microbiology, plant-insect interactions, metabolomics, histological analysis of insect tissues and bacteria (microscopy and flow cytometry).
The project can be pursued in the frame of a PhD project that would focus on the detailed analysis of *Burkholderia* functions involved in the establishment of the symbiosis with stinkbugs.

Bibliographie *:*

Sélection d'autres publications de l'équipe sur le sujet:

Ohbayashi et al 2019. Comparative cytology, physiology and transcriptomics of *Burkholderia insecticola* in free-living state and in symbiosis with the bean bug *Riptortus pedestris*. *ISME J.* 13, 1469-1483.

Ohbayashi et al 2015. Insect's intestinal organ for symbiont sorting. *Proc. Natl. Acad. Sci. U.S.A.* 112, E5179-E5188.

Raina et al 2019. The role of microbial motility and chemotaxis in symbiosis. *Nat. Rev. Microbiol.* 17, 284-294.

Takeshita and Kikuchi 2017. *Riptortus pedestris* and *Burkholderia* symbiont: an ideal model system for insect-microbe symbiotic associations. *Res. Microbiol.* 168, 175-187.

Ce stage peut-il se poursuivre par une thèse ? : oui