

M2 INTERNSHIP SUBJECT

TITLE: Co-occurrence and interactions between *Fusarium* and wheat-associated bacteria.

CONTEXT:

Crops production is frequently impaired by plant diseases such as Fusarium Head Blight (FHB). This disease is the result of the infection of wheat spikelet tissues by pathogenic *Fusarium* species, resulting in the reduction not only in grain yield but also in grain quality due to the production of mycotoxins by the pathogen.

FHB is known as a disease complex since more than one species from the genus *Fusarium* is involved in the infection. It is clearly acknowledged that FHB incidence and *Fusarium* species composition are highly correlated with climatic conditions, agronomic factors and host genotype. However, the role of the wheat microbiota on the success of the *Fusarium* infection is less known and needs further investigations.

OBJECTIVES:

The main objective of this internship is to determine whether and how the wheat microbiota influences the species composition of *Fusarium* in wheat grains. The main hypothesis of the project is that wheat-associated bacteria play a role in shaping the composition of *Fusarium* species in wheat grains and therefore the levels and profile of mycotoxins. To validate this hypothesis, we propose a new approach combining molecular and culture-based methods. The goal is not only to identify taxa that co-occur within wheat grains, but also to test their interactions *in vitro*.

METHODS:

One part of the internship will consist in analysing bacterial diversity in a large dataset obtained via sequencing of the 16S rRNA gene in grain samples from soft and durum wheat (N = 504 samples). These cereals were sampled from different regions in France, representing various agricultural practices and climatic conditions. A comprehensive set of metadata is available for each sample (e.g. wheat variety, weather conditions, agronomic practices, mycotoxin concentrations, infecting *Fusarium* species). The influence of these factors on alpha- and beta-diversity of bacterial communities will be assessed by investigating species richness, diversity indexes and the relative abundance of individual taxa via univariate (e.g. linear mixed effect models) and multivariate comparisons (e.g. PERMANOVA). Unconstrained and constrained ordination of microbiome read count data will be also performed by using Principal Coordinates Analysis (PCoA), Canonical Correspondence Analysis (CCA), as well as other algorithms such as the Row-Column interaction model of dimension M (RC(M)) developed by Hawinkel et al. (2019).

Co-occurrence network analysis will be performed by integrating the results of the sequencing of bacterial communities and the diversity of *Fusarium* species that is currently investigated for the same sample set in the frame of the EvolTox project (ANR-20-CE32-0011). The construction and comparison of networks will be performed by using existing tools such as NetCoMi (Peschel et al., 2021), which was developed to measure associations between taxa, to analyse network topology and to perform differential association analysis. Association between bacterial taxa and the mycotoxins

la science pour la vie, l'humain, la terre

UR1264 MycSA

71 avenue Edouard Bourlaux

CS2032

33882 Villenave d'Ornon – Cedex

France

Tel.: +33 1 (0)5 57 12 24 75

Rejoignez-nous sur :

X : @INRAE_MYCSA

LinkedIn : INRAE_MYCSA

<https://mycsa.bordeaux-aquitaine.hub.inrae.fr/>

www.inrae.fr

analysed in the project EvolTox will be also tested in order to determine the specificity of bacterial taxa to mycotoxins (Darcy et al., 2022).

Finally, bacterial synthetic communities (SynComs) representative of the wheat microbiota will be assembled and their interactions with several *Fusarium* species will be evaluated in vitro. The bacterial isolates and SynComs will be co-cultured with *Fusarium* according to the network correlations, either as single isolate or as SynComs. Bacterial and fungal growth will be followed for each co-culture by qPCR, and the production of mycotoxins will be assessed by HPLC-DAD and LC-MS.

PREREQUISITES:

Candidates should have a strong background in microbial ecology and/or bioinformatics, with a good command of bioinformatics tools and the ability to work in a microbiology lab. Interest in plant microbiology would be an advantage.

REFERENCES

Hawinkel, S., Kerckhof, F.-M., Bijmens, L., Thas, O., 2019. A unified framework for unconstrained and constrained ordination of microbiome read count data. PLOS ONE 14, e0205474. <https://doi.org/10.1371/journal.pone.0205474>

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Darcy, J.L., Amend, A.S., Swift, S.O.I., Sommers, P.S., Lozupone, C.A., 2022. specificity: an R package for analysis of feature specificity to environmental and higher dimensional variables, applied to microbiome species data. Environ. Microbiome 17, 34. <https://doi.org/10.1186/s40793-022-00426-0>

KEYWORDS: plant microbiota, metabarcoding, co-occurrence networks, synthetic communities, mycotoxins

NAMES OF THE SUPERVISORS: Louis Carles

NAME and ADDRESS OF THE TEAM/LABORATORY: UR 1264 MycSA, 71 avenue Edouard Bourlaux – CS 2032, 33882 Villenave d'Ornon Cedex (France)

TEL: +33 5 57 12 24 90

E-MAIL: louis.carles@inrae.fr

INRAE

la science pour la vie, l'humain, la terre

UR1264 MycSA

71 avenue Edouard Bourlaux

CS2032

33882 Villenave d'Ornon –Cedex

France

Tel.: +33 1 (0)5 57 12 24 75

Rejoignez-nous sur :

X : @INRAE_MYCSA

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