

## XoCo

# Xanthomonas Oryzae Co-infections : developing methodologies to address their epidemiological consequences.

## ABSTRACT

The composition and impact of microbial communities in virtually any conceivable ecosystem on earth are being unravelled at a fast pace in large part because of the development of short read NGS technologies for amplicon sequencing. However, despite its importance the composition of diseased plant tissue microbial assemblages in nature still remains poorly characterized and would probably require more resolute approaches for taxonomic classification. Functional interactions between pathogens infecting the same eukaryotic host have been recorded in many systems including plants. Data in the literature notably provides evidence that related genotypes belonging to a genus encompassing pathogenic bacteria can mutually influence their fitness and disease outcomes.

Our group at UMR PHIM is interested in various aspects of rice foliar diseases caused by bacteria belonging to the *Xanthomonas oryzae* (Xo) species that represent the fifth most damaging pest or pathogen on rice. Our preliminary data stemming from disease surveys in Africa and Asia suggests that Xo populations in single symptomatic leaves are unexpectedly polymorphic and that bacteria belonging to different phylogenetic subgroups can co-exist within the infected tissue. Xo co-infections raise a number of questions with regard to disease epidemiology and control.

The XoCo project aims at establishing the methodological foundation for systematically addressing these questions in rice cultivation with an unprecedented genomic resolution. For this, because the last generation long reads sequencing platform Oxford Nanopore Technologies (ONT) offers unmatched capabilities for shotgun metagenome sequencing with subspecies resolution, we propose to capitalise on our previous experience with ONT to develop an expertise on ONT-based methodologies for the description of co-infecting Xo bacterial genomes variability inside a rice leaf sample. Specifically, we will first investigate the effect of mixed versus single inoculation on disease symptoms intensity and the multiplication of the bacterial protagonists in the lab using a few pairs of relevant co-inoculated Xo strains. The rice leaf material generated at this stage will be used as an input to test original approaches and define optimal experimental procedures for ONT sequencing of bacterial DNA from diseased rice leaves. Finally, benchmarking computational tools to conduct metagenome assembly and read taxonomic classification on this sequencing dataset will define if and under which conditions, we achieve intraspecific resolution.

Altogether, XoCo output should provide the methodological foundation for future research on the dynamics of the plant bacterial pathobiome in the field. Ultimately, it could serve as a reference for long read shotgun metagenomic sequencing at small microgeographic and evolutionary scales of both environmental and laboratory samples in the Agropolis community.

**Keywords :** Genomic Epidemiology, Pathobiome, Oxford Nanopore technologies, Bacterial disease, Rice

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**Project leader's institution :** IRD

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## GOAL

We are interested in rigorously assessing the actual prevalence of field Xo Co-infections. It would be desirable to discriminate the colonizing bacteria at an infraspecific level and generate Xo leaf metagenome assemblies to decipher metapopulation dynamics and the consequences of co-infection on the fitness of the individual genotypes. It is also critical to address the potential consequences of co-infection on resistance genes efficacy.

With the long term goal to address the prevalence, the ecological relevance and the epidemiological consequences of non- clonal rice leaf Xo infections, the XoCo project primarily aims at establishing the methodological framework necessary for this endeavor by first exploring a range of experimental parameters at a small scale in the laboratory. In a nutshell, the general objective is to develop expertise on ONT-based methodologies for the description of co-infecting Xo bacterial genomes variability inside a rice leaf sample.