

Proposition de sujet de stage M1 (S2 2025)

Laboratoire d'Ecologie Microbienne (LEM)



Université Claude Bernard



Title: Development of molecular tools (metabarcoding) for the study of soil actinobacteria, focusing on the genus *Frankia*

Keywords: molecular tool development, metabarcoding, *Frankia*, actinobacteria, soil, actinorhizal symbiosis

Laboratory:

UMR 5557 Laboratoire Ecologie Microbienne (LEM) ; <https://www.ecologiemicrobiennelyon.fr/>
Equipes d'accueil : Symbiose Actinorhizienne (SYM)/ Bacterial Efflux and Environmental Resistance (BEER).
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Actinobacteria play key role in soil and plant health (Bhatti et al 2017). Indeed, they play essential role in biogeochemical processes by fixing atmospheric nitrogen or in the carbon cycle by possessing numerous hydrolytic enzymes as saprophytic microorganisms. They also interact with various organisms (plants, insects, humans, etc...) as beneficial (*Frankia*, *Bifidobacterium*, *Streptomyces*, *Micromonospora*), commensal (*Propionibacterium*) or pathogenic (*Nocardia*, *Mycobacterium*, *Rhodococcus*,...) organisms. Among them, *Frankia* genus are able to establish symbiosis with actinorhizal plants (more than 260 species belonging to eight families). Actinorhizal plants represent a model of choice that has yet to be fully explored in the field of revegetation), in a variety of contexts (urban environments, reforestation, rehabilitation of degraded sites, etc.). Thanks to their capacity to "fix atmospheric nitrogen" via the symbiosis they establish in the root system with the actinobacterium *Frankia*, these plants are described as pioneer plants capable of colonizing depleted and contaminated soils, and playing an important ecological role in the early stages of plant succession (Hu et al 2023). Mostly trees or shrubs, they make a major contribution to soil fertility, enriching the soil with nutrients by degrading roots and leaf litters.

Despite the importance of this symbiosis, the relationships between the symbiotic *Frankia* populations (present in the alder nodules) and those of the soil are poorly understood, whereas the very success of revegetation operations requires a better understanding of these relationships, since the alder recruits its symbionts from the *Frankia* pool in its rhizosphere. This lack of knowledge is due to the scarcity of molecular tools available for studying *Frankia* (and more broadly actinobacteria) in the soil. Several constraints are due to the fact that *Frankia*, like other actinobacteria, have highly conserved housekeeping genes (>99 %), GC-rich genomes (>70%), dormant population making it more challenging to study their diversity via metabarcoding approaches.

The aim of the internship is to identify candidate genes that will be used in metabarcoding approaches to detect and quantify actinobacteria and more specially *Frankia* populations present in soil. This work will be based on a search in existing databases (>30 *Frankia* genomes, >50 Actinomycetia genomes) in order to identify candidate genes (genes found in all *Frankia* and sufficiently discriminating to distinguish between

different populations). Because of their beneficial role in soil and plant health, the trainee will also develop similar tools for the more global study of soil actinobacteria.

Technically and from the different genomes, the student will have to identify the core-genome. A number of genetic markers will be selected according to different criteria, such as their specificity in characterising one or all of the genera represented in the class of Actinomycetia, the presence of conserved and specific domains, their phylogenetic divergence, etc... Then, primer design methods will be according to De Silva pipeline (Da Silva et al 2024). High-quality (well covered) in-house and database *Frankia* genomes will be aligned based on coding regions with BLAST, and hits will be considered only if they are detected in the target genomes but absent from the genomes included in the RefSoil database (Dunivin et al 2019). For the Actinomycetia (class), we will query against a curated collection of genomes available NCBI or GOLD database entries. The student will be responsible for curating reproducible bioinformatic pipelines from existing tools and performing *in silico* validation.

Profile required: M1 Bioinformatics student. Genomic data analysis, knowledge of prokaryotic genomic databases, theoretical basis in metabarcoding, knowledge of strategies for effective primer design.

References:

Bhatti AA, Haq S, Bhat RA (2017). Actinomycetes benefaction role in soil and plant health. *Microb Pathog* **111**: 458-467.

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Hu B, Flegmetakis E, Liu Z, Hänsch R, Rennenberg H (2023). Significance of nitrogen-fixing actinorhizal symbioses for restoration of depleted, degraded, and contaminated soil. *Trends in Plant Science* **28**: 752-764.