



Illustration d'un pock. Un pock (flèche) résulte du transfert d'éléments conjuguatifs entre une souche donneuse de *Streptomyces* (au centre du pock) vers une souche réceptrice (tapis bactérien). Ce transfert induit un retard de croissance chez les réceptrices, permettant de visualiser à l'œil nu les événements de conjugaison dans une population de *Streptomyces*.

Microbiota Experimental Evolution under plant selection

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Thematic actions concerned: WP1 / Transversal WP2

Context —

It is now increasingly recognized that the health and resilience of trees and forests crucially depend on their associated microflora. By studying bacteria from a plant microbiota, we showed that these latter can massively exchange genes by horizontal transfers at the population level¹. If those fast-evolving processes are a key to adaptation, little is known, on the other hand, regarding the biotic factors that influence them.

Objectives —

This project aims to decipher the evolutionary and adaptative responses occurring between plants and their microbiota. This will shed light i) on gene fluxes within a bacterial population during several generations on the genomic changes (e.g. gene fluxes) occurring in a bacterial population under the plant selection and ii) the pay back return that could benefit the plant from these evolutionary processes.

Approaches —

We will mimic evolutionary processes with evolution experiments that consist in propagating microorganisms under controlled conditions over many generations. These experiments will be performed using a *Streptomyces* rhizospheric population under the selective pressure of plants or their exudates. After several generations, evolutionary innovations will be detected by genomic comparisons of the evolved populations with the ancestral strains. The impact of these processes on plant health (growth promotion, pathogen inhibition, mineral solubilization...) will be tested with PGP (Plant Growth Promoting) tests.

Key results — (presented as separated bullet points)

- Labelling of strains for monitoring in long-term experiments
- Identification of the conditions for conjugative transfer (culture media, genotypes)
- Demonstration of massive conjugative transfer in experiments (between 200 kb and 2Mb representing 2 to 15% of their genome are transferred during a single event)
- Setting up of PGP tests between *Streptomyces* and poplar
- Sequencing of first strains from evolution experiments

Main conclusions including key points of discussion —

The experimental evolution experiments required the establishment of optimal experimental conditions and an appropriate panel of strains to ensure their success. Preliminary results on bipartite conjugations showed that gene transfer was substantial and could involve a transfer of up to 10% of the genome during a conjugation event. A first experimental evolutionary experiment allowed the selection of evolved strains that recombined with each other (selection by exchange of antibiotic resistance markers). The genome of these strains is currently being analysed and will make it possible to estimate the evolutionary consequences of a population exchanging genes over several generations

Perspectives —

This project will (i) estimate gene flow in a bacterial population over several generations, (ii) determine whether the plant stimulates genetic exchange in rhizosphere communities, (iii) test whether the plant chooses a particular genotype or rather a diverse population, and (iv) test whether the evolved bacterial strains provide an advantage, individually or collectively, to the plant in terms of health and growth

Leveraging effect of the project—

This overall project will shed light about the interplay existing between plants and their microbiota, a key factor regarding the forest functioning. It will also lay the foundations for future research and collaborations based on experimental evolution in the DynAMic laboratory. As an extension of this work, a thesis project has been accepted (LUE in collaboration with the LCP-A2MC laboratory, Metz).

¹ Tidjani et al. 2019, mBio. 2019 Sep 3;10(5)