



*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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Actions thématiques concernées : 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 recently 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 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 conjugative transfer in bipartite experiments
- Demonstration of significant chromosomal material transfer between two efficient strains for conjugation: between 200 kb and 2Mb representing 2 to 15% of their genome (12 Mb)

Main conclusions including key points of discussion —

Experimental evolution experiments require prior experimental optimisation to ensure that they run successfully and that the results are sustained over a period of several months. We have established these different conditions, as well as an appropriate panel of strains. Preliminary results on bipartite conjugations have established that gene transfer is substantial and can involve a transfer of up to 10% of the genome during a conjugation event. The experimental evolution experiment *sensu stricto*, which will run continuously over several months, will be launched in early 2022 and will be completed in the first half of that year.

Perspectives —

This project will enable (i) to determine whether the plant stimulates genetic exchanges among the rhizospheric community, (ii) to test if the plant rather selects a particular genotype or a diverse population, and (iii) to 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, co-financing and thesis projects are being submitted (respectively INRAe MICA department and LUE in collaboration with the LCP-A2MC laboratory, Metz).

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