

## Microbiota Experimental Evolution under plant selection

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*Thematic action concerned:* Transversal WP2

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### **Context** —

It is now recognized that the health and resilience of trees and forests depend on their microflora. By studying bacterial populations in a plant microbiota, we have shown that they evolve through gene transfer<sup>1</sup>. However, little is known about the extent, timing and consequences of these transfers, the biotic factors that influence them and their adaptive consequences.

### **Objectives** —

This project aimed to decipher the evolutionary and adaptive responses taking place between plants and their microbiota. The objectives were to better understand i) the genomic innovations (e.g. gene flow) occurring in a rhizospheric bacterial population and ii) the benefits that the plant can derive from a diverse bacterial population. This project aims to decipher the evolutionary and adaptive 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** —

Evolutionary processes were mimicked in conjugation experiments and in experimental evolution experiments involving the cultivation of microorganisms over several generations. Evolutionary changes were detected by genomic comparison between evolved and ancestral strains. The impact on plant health of populations compared to single strains was tested using PGP (Plant Growth Promoting) tests on plants.

### **Key results** —

- Labelling of strains for monitoring in long-term experiments
- Identification of the conditions for conjugative transfer (culture media, genotypes)
- Demonstration of massive conjugative transfer between strains in a population (2 to 30% of the genome is transferred during a single event)
- Modification of molecule biosynthesis genes in 90% of conjugators compared with parental strains
- Establishment of PGP tests between *Streptomyces* and poplar

### **Main conclusions including key points of discussion —**

Our results on bipartite conjugations showed that, during a single conjugation event, gene transfer was massive between *Streptomyces* and that this evolutionary process led to a modification of biomolecule biosynthetic pathways in over 90% of recombinant strains. An initial experimental evolution experiment to mimic these evolutionary processes over several generations failed to observe any additional effects compared with simple conjugation, but did enable us to refine the choice of strains and experimental conditions for further experiments. These advanced strains could subsequently be tested in interaction tests with poplars, for which co-culture protocols with several *Streptomyces* strains were developed during this project. Our results have shown that gene transfer has a major impact on the metabolic diversification of strains from the same population, and thus contributes to rapid adaptation in the rhizosphere in association with the plant.

### **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 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 has also laid 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). publication on our initial results is currently being drafted.

<sup>1</sup> Tidjani et al. 2019, mBio. 2019 Sep 3;10(5)