



Comment déceler la conjugaison à l'œil nu ?

Sur un tapis de bactéries réceptrices, les 'pocks', ces figures circulaires (4-5 mm), révèlent les événements de conjugaison avec la donneuse (au centre du pock). Le pock résulte du retard de croissance induit par la diffusion d'un élément conjugatif de la donatrice vers la réceptrice. Le circo traduit la cartographie des transferts (formation de 'pocks') au sein de la population de *Streptomyces*, et révèle que seuls certains isolats donnent et/ou reçoivent les éléments conjugatifs : la population n'est pas panmictique.

Interplay between lateral gene transfer in bacteria and rhizosphere functioning

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Thematic actions concerned: WP2 (principal) et WP1

Context —

The plant microbiota has a major role in the health of trees by participating, for example, in their growth or defence against pathogens. Generally speaking, the more diverse a microbiota is, the more effective it is for its host. Results suggest that one level of organization of this diversity could be at the level of bacterial populations, i.e. in sister strains that interact with each other in the niche, but with potentially different capacities. These variable functions, linked to the genetic diversification of the population, would lead to the production of public goods and synergistic action. In return, the plant's root exudates would influence the microbiota by increasing the frequency of gene transfer and enable the emergence of new functions within the population.

The first objective of this project is to measure the effect of the rhizosphere on gene transfer and its consequences on the diversification of a *Streptomyces* population. The second is to compare the influence and efficiency of *Streptomyces* populations compared to single isolates for functions related to tree health and growth.

Approaches —

A population of *Streptomyces* from a tree microbiota is available in the laboratory. The genome of some strains is sequenced and comparison of these strains has revealed a great genetic and potentially functional diversity. Conjugative elements have been associated with the genesis of this diversity (Tidjani *et al.*, 2019). In *Streptomyces*, their transfer results in growth retardation of the recipient strain observable with the naked eye in a Petri dish (see

illustration). This phenomenon will enable to quantify directly the influence of the plant (rhizosphere, exudates) on the intensity of gene transfer within the population. The conjugative elements of *Streptomyces* could transfer other portions of the genome concomitantly with their own transfer. In order to see if this mechanism is at the origin of the high genetic diversity observed in our population, conjugations will be performed with strains labelled at different chromosomal loci and the transconjugates will be analyzed by resequencing. Concerning the second objective, our strains will be tested in interaction with root systems or in systems mimicking the rhizosphere for the direct or indirect growth of model plants (root development, mineral solubilization, pathogen inhibition). For these different functions, we will compare the potential synergistic effect of the population compared to the strains alone.

Key results —

- A complete inventory of conjugative elements was carried out: several bioinformatic methods (including conj-scan, ICE-finder, search for a set of label genes) were applied and revealed the presence of 52 elements that could be grouped into 26 distinct families. None of the elements are shared by all individuals, and 15 of them are present in only one individual. In addition, defective elements (lacking one of the label genes) were characterized. The results show that the elements are very dynamic within the rhizosphere.
- A mapping of the sequenced strains capable of conjugating with each other (121 pairs) was carried out. Fifteen of the 110 crosses are visibly productive (pock formation) with transfer efficiencies between 20% and 100% (i.e. between 20 and 100% of donors form a pock).
- While the conjugative elements characteristic of actinomycetes are dependent on a single TraB protein for their transfer, we have identified a T4SS-dependent element. The latter is currently being labelled to test its functional character.
- Different productive conjugation couples have been selected and the donor and recipient strains differentially labelled in order to measure the extent of chromosomal marker transfer during conjugative transfer. Recombinant formation has been obtained at frequencies of the order of 10^{-5} (per donor); recombinants are being validated for full sequencing. Comparison of their genome with that of the parental strains will make it possible to identify the background on which recombinants are formed, and to determine the size - and possibly the number - of transfer events.

Perspectives —

The sanitary situation has considerably disturbed the progress of the experiments, in particular the confinement in March led to the loss of material (strains under construction), and the degraded functioning of the laboratories since the exit of the confinement does not favour the animation of the thesis. Finally, the current development of the epidemic has redirected the high throughput sequencing facilities of the Institut Pasteur platform (IntegraGen) towards the sequencing of covid-19 variants, leading to a probable additional delay in data acquisition. This incident occurred after the closure of our previous service provider (I2BC platform, Gif-sur-Yvette). The extension of thesis funding by ARBRE, the INRAE MICA department and the GE region (in progress) will hopefully allow us to mitigate the impact of the crisis we are going through.

The collaboration with Claire Fourrey (IAM) in order to test the potential influence of the tree (poplar) on the transfers and reciprocally the impact of *Streptomyces* on their growth is still an objective of the thesis. The sanitary situation shifted this objective towards the end of the experimental work. Ultimately, this project will lead to a better understanding of the existing interconnections between trees and their microbiota, a crucial parameter for the homeostasis of the forest ecosystem and its resilience.

Caroline Choufa's thesis was able to continue into the 5th year thanks to a half-ATER position in microbiology. The PhD defence is scheduled for the end of the academic year 2023 at the latest. A first paper has been published "Prevalence and mobility of Integrative and Conjugative Elements within a *Streptomyces* natural population" Choufa C, Tidjani AR, Gauthier A, Harb M, Lao J, Leblond-Bourget N, Vos M, Leblond P, Bontemps C. *Front Microbiol.* 2022 Sep 13;13:970179. doi: 10.3389/fmicb.2022.970179. PMID: 36177458; PMCID: PMC9513070.

We are also working on the exploitation of comparative genomics results on chromosomal DNA transfers accompanying that of conjugative elements. These trans transfers are massive and had never been revealed other than by formal genetics by David Hopwood (1970).

Another article is currently upon writing based on these results. We plan to publish them in journal with a large audience because of the originality and dramatic nature of these DNA flows. No transfer of this magnitude has been described since the transfer of factor F in *Escherichia coli*, described in the 1960s by F. Jacob and William Hayes. A first version of the article will be published shortly in bioRxiv.