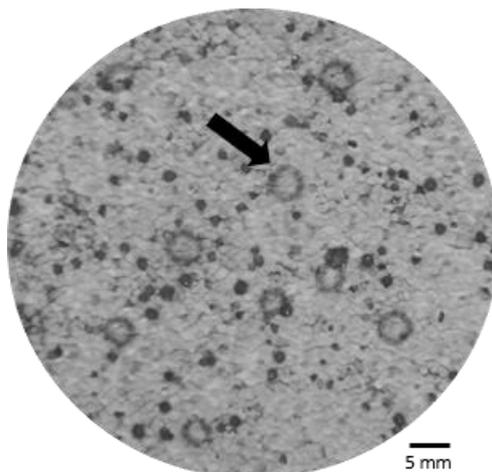


GENTRHI



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.

Interplay between lateral gene transfer in bacteria and rhizosphere functioning

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LabEx partners:: UMR Interactions Arbres/Micro-organismes (IAM)

With the collaboration of : Claire Fourrey (IAM) et Michiel VOS (Uni. Exeter)

Work packages: WP2 (principle) -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 the conjugative elements was carried out: several bioinformatics methods based on label search (HMM profiles, Blast) or dedicated tools (IceScreen produced in the laboratory) revealed the presence of 51 elements that can be grouped into 19 distinct families (based on the TraB label). None of the elements is shared by all individuals, and 14 of them are present in only one individual. In addition, 8 defective elements (lacking one of the tag genes) were characterized. The ensemble shows that the elements are very dynamic within the rhizosphere.

- **The *Streptomyces* population is not panmictic.** A mapping of the sequenced strains capable of conjugating with each other (121 pairs) was performed. Thirteen of the 110 crosses are visibly productive (pock formation) with variable transfer efficiencies (between 20% and 100%, i.e. between 20 and 100% of donors form a pock). It seems that there are barriers to transfer within the population; transfer barriers or maintenance of acquired DNA? The analysis of the genomes continues to look for functions potentially involved in these barriers (restriction-modification systems, CRISPR, ...).

- **Identification of ICE (Integrative and conjugative element).** While the conjugative elements characteristic of actinomycetes are dependent on a single TraB protein for their transfer, we have identified two T4SS-dependent elements called ICE for Integrative and conjugative element; one plasmidic, the other chromosomal. We have marked and studied the chromosomal element: it excised from the chromosome showing its functional character, but no transfer was detected under our experimental conditions.

- **Massive conjugative transfer of DNA.** Different productive conjugation pairs were selected and donor and recipient strains differentially labeled in order to measure the extent of chromosomal marker transfer during conjugative transfer. Recombinant formation was obtained at frequencies on the order of 10^{-5} (per donor); recombinants are being validated for full sequencing. The results are currently being processed. However, genome analysis of the first recombinants ($n=12$) by searching for SNPs (Single Nucleotide Polymorphism) specific to one or the other of the parental strains along the chromosome, allows us to conclude that there are massive transfers of chromosomal DNA between a strain possessing a conjugative element and a strain that lacks it. Thus, in the cross studied, the sum of the size of the fragments of the donor genome identified in the genome of the recombinants varies between 2% and 20%, i.e. between 217 kb and 2,190 kb, distributed between 3 and 20 DNA fragments of significant size (threshold used, 1 kb). This phenomenon is reminiscent of DCT, Distributive Conjugative Transfer, highlighted in *Mycobacteria* (Gray et al., 2018), but with an exceptional magnitude.

Conclusion

The original biological system constituted by the environmental population of *Streptomyces* is ideal to test several hypotheses on transfers in soil.

A first article is being finalized for *Frontiers in Microbiology* 'Prevalence and mobility of Integrative and Conjugative Elements within a *Streptomyces* natural population' Choufa et al.

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

We plan to publish these data in a mainstream journal because of the originality and spectacular 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.

Perspectives —

The sanitary situation has considerably disrupted the progress of the experiments, in particular the confinement of March 2020 has led to the loss of material (strains under construction), and the degraded functioning of the laboratories since the end of the confinement does not favor the animation of the thesis. Finally, the current development of the epidemic has redirected the high throughput sequencing resources of the Institut Pasteur platform (IntegraGen) towards the sequencing of covid-19 variants, leading to a probable additional delay in data acquisition.

The collaboration with Claire Fourrey (IAM) in order to test the potential influence of the tree (poplar) on the transfers and conversely the impact of *Streptomyces* on their growth is still an objective of the project. The sanitary situation

has shifted this objective towards the end of the experimental work. Ultimately, this project will allow 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 4th year thanks to a half-ATER position in microbiology. Caroline's work has been the subject of several communications in national and international conferences, and a first publication is in progress. A second one will focus on trans conjugative transfers. The defense is planned for the end of the academic year at the latest.

Gray TA, Derbyshire KM. Blending genomes: distributive conjugal transfer in mycobacteria, a sexier form of HGT. *Mol Microbiol.* 2018 Jun;108(6):601-613.

Tidjani A-R, Lorenzi J-N, Toussaint M, van Dijk E, Naquin D, Lespinet O, Bontemps C, Leblond P. 2019. Massive Gene Flux Drives Genome Diversity between Sympatric *Streptomyces* Conspecifics. *mBio* 10.