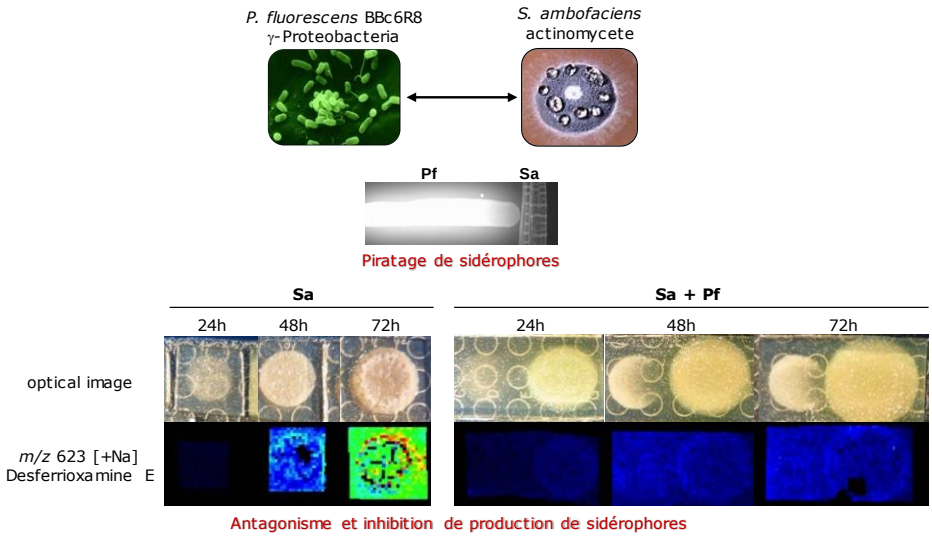
# CoMicSoFt




## Towards the understanding of microbial dialogues within forest soil ecosystems: study of the metabolic exchanges between *Streptomyces* and *Pseudomonas*.

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**Context** — Within the microbial communities, microorganisms communicate and manipulate their environment and neighboring microbial populations in a process known as metabolic exchange. These metabolic exchanges or dialogues are key processes in the structuration of forest soil microbial communities, resilience of these communities in response to external perturbations (e.g. climate changes...) and plant development and health. Nevertheless, little is known about the chemicals and molecular actors involved in these metabolic exchanges.

**Objectives**— We aim to decipher these microbial dialogues and the actors involved in these dialogues by studying the interactions within a model couple of soil bacteria: *Pseudomonas fluorescens* BBc6R8 and *Streptomyces ambofaciens* ATCC23877. Pseudomonas and Streptomyces are common bacteria in temperate forest ecosystems.

**Approach**— Our approach within the CoMicSoFt project consisted in the identification by spectrometric approaches (MALDI-TOF IMS and nanoDESI, collaboration with Pieter C Dorrestein, UCSD, USA) of the chemicals involved in the dialogue between the two bacterial partners and more globally in the analysis of the pairwise interaction impact on the secondary metabolism of these two bacterial species.

Co-cultures between *P. fluorescens* BBc6R8 and different *S. ambofaciens* mutant strains (available in the laboratory) deficient for the biosynthesis of secondary metabolites (including antibiotics) were planned to determine the impact and role of these metabolites on the pseudomonad.

**Key results** — The interaction studies *Pseudomonas* – *Streptomyces* were carried out on different growth media and the observed phenotypes revealed that:

- Under iron limitation (R2 medium), *P. fluorescens* is able to take up the desferrioxamine and coelichelin siderophores produced by *S. ambofaciens*. This would explain why, when in co-culture

with *S. ambofaciens*, the expression of the siderophore biosynthetic and regulatory genes is not induced is not induced in *P. fluorescens*.
- *P. fluorescens* BBc6R8 inhibits the production of kinamycin by *S. ambofaciens* by interfering with the regulatory cascade controlling the biosynthesis of the antibiotic. This inhibition is likely due to a quorum quenching mechanism.
- *P. fluorescens* BBc6R8 also prevents the production of the γ-actinorhodin antibiotic by *Streptomyces coelicolor*. However, this inhibition is due to the production of gluconic acid by *Pseudomonas* and consequently to the acidification of the culture medium.

Spectrometric analyses by IMS and naoDESI have shown other interesting and unexpected phenotypes:
- *P. fluorescens* BBc6R8 stimulates the production of acyl-desferrioxamines (R2 medium, nanoDESI). These derivatives of desferrioxamines have been only discovered in *S. coelicolor* when in interaction with other actinomycetes (Traxler et al., 2013).
- More surprisingly, the effects of the interactions on the siderophore production were dependent on the culture media and completely opposite according to the growth conditions. Thus on KB medium; *P. fluorescens* prevents the siderophore production in *S. ambofaciens* (IMS analyses) and inhibits the growth of the actinomycete.

**Main conclusions including key points of discussion** — Our results show that the two bacteria influence strongly the secondary metabolism of their partner. The mechanisms responsible for the observed effects are diverse such as quorum sensing or the ability to use the secondary metabolites produced by the partner. In addition, the observed phenotypes for a same family of metabolites, *i.e.* the siderophores, suggest that the relationships between the partners are variable according to their environment (here the culture medium).

**Future perspectives** — The aim is now to correlate these spectrometric analyses to a global transcriptional analysis (RNAseq) that will be undertaken from co-cultures done in the same conditions than the ones used for the study of the metabolic exchanges. This will allow the identification of the signaling molecules and of the molecular actors involved in these metabolic exchanges. The mechanism by which the BBc6R8 strain induces the acyl-desferrioxamine siderophores will be studied.

**Valorisation** —

**Articles**

Deveau A, Gross H, Palin B, Mehnaz S, Schnepf M, Leblond P, Dorrestein PC and Aigle B (2016) Role of secondary metabolites in the interaction between *Pseudomonas fluorescens* and soil microorganisms under iron-limited conditions. FEMS Microbiol Ecol, 92(8).

Galet J, Deveau A, Hôtel L, Frey-Klett P, Leblond P and Aigle B (2015) *Pseudomonas fluorescens* pirates both ferrioxamine and ferricoelichelin siderophores from *Streptomyces ambofaciens*. Appl Environ Microbiol, 81(9):3132-41.

Galet J, Deveau A, Hôtel L, Leblond P, Frey-Klett P and Aigle B (2014) Gluconic acid-producing *Pseudomonas sp.* prevent γ-actinorhodin biosynthesis by *Streptomyces coelicolor A3(2).* Arch Microbiol, 196(9):619-27.

**International communication**

Deveau A (2016). Bacterial Fungal interactions in forest soils. Workshop “Fungal-Bacterial Interactions”, Dec 2016, Neuchatel, Suisse (Oral presentation)

Deveau A (2016) Application of Imaging Mass Spectrometry to the analysis of the interaction between fungi and bacteria. Fungal-bacterial interaction workshop, ECFG, Apr 2016, Paris, France (Oral presentation)

Deveau A (2015). From mutualism to antagonism: iron acquisition during microbial interactions. Soil Microbial Ecology Conference, Dec 2015, Praha, Czech Republic (Oral presentation).

Galet J, Deveau A, Leblond P, Frey-Klett P and Aigle B (2013) Towards the understanding of microbial dialogues within soil ecosystems: studies of the interaction between *Streptomyces ambofaciens* and *Pseudomonas fluorescens*. SGM Spring conference, Manchester, UK. (Poster)

Galet J, Deveau A, Leblond P, Frey-Klett P and Aigle B (2012) Biotic interactions between two soil bacteria,*Streptomyces ambofaciens* and *Pseudomonas fluorescens*. Symposium Actinobacteria within soils: capacities for mutualism, symbiosis and pathogenesis, Münster, Germany. (Poster)

National communication (oral presentations)

Galet J, Deveau A, Hôtel L, Leblond P, Frey-Klett P and Aigle B (2013) Interactions entre deux bactéries du sol, *Streptomyces ambofaciens* - *Pseudomonas fluorescens*. 9$^{e}$ Congrès National de la Société Française de Microbiologie, Lille, France.