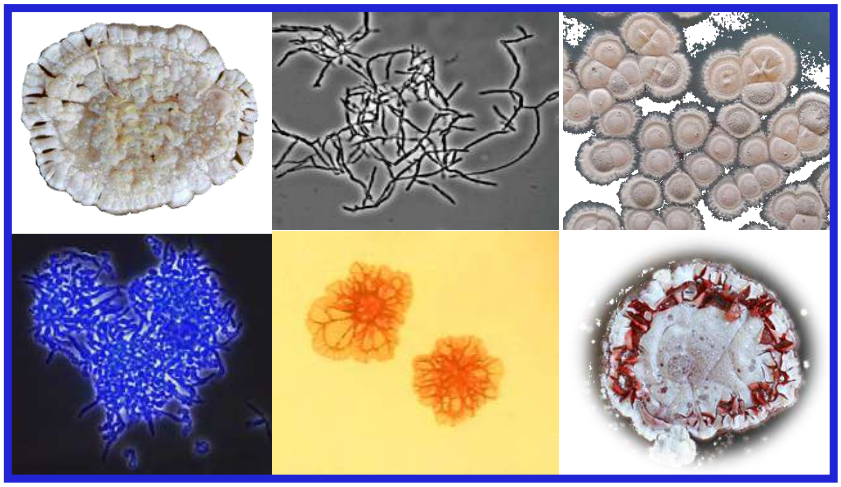
ACTINO 2020

Journées des actinobactéries

7 et 8 Décembre 2020

En Visio, via PremC



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Programme

Lundi 7 décembre

9h00-9h15 **Ouverture et Introduction**

Session 1 : **Génomique et Evolution**

Modérateur Jean-Luc Pernodet

Posters : **Flash Présentations**

Modérateur Hervé Leh

12h05-12h20 Q & A

12h20-14h00 *Déjeuner*

Lundi 7 décembre

Session 2 : **Métabolisme spécialisé et biologie de synthèse**

Modératrice Sylvie Lautru

Mardi 8 décembre

Session 3 : **Interactions actinobactéries/plantes**

Modérateur Bernard Dumas

Mardi 8 décembre

Session 3 : **Interactions actinobactéries/plantes**

Modérateur Bernard Dumas

Session 4 : **Régulation**

Modératrice Soumaya Najah

Résumés de communications

(par ordre de passage)

Gene flux and chromosomal recombination as key drivers of adaptability and population functioning of *Streptomyces*

Pierre Leblond, Université de Lorraine, INRA, DynAMic, Nancy, France.

Unlike most bacteria, *Streptomyces* have a linear chromosome (6-12 Mb). The chromosome arms are highly recombinogenic, promoting the formation of large rearrangements, including deletions that can cause the loss of up to a quarter of the chromosome in a single event, and high intensity gene amplifications. While these rearrangements have revealed the compartmentalization of the genome with essential genes at the center of the chromosome and dispensable genes in the arms, the contribution of genome rearrangements to the evolutionary mechanisms and biology of the organism has long been debated. Comparative genomics approaches have recently demonstrated that increased recombination in the chromosomal arms contributes to genetic diversification both at the level of the bacterial population (Tidjani et al., 2019, 2020) and of the bacterial genus as a whole (Lorenzi et al., submitted). At the genus level, diversification involves an increasing gradient of horizontal transfer and genomic rearrangement events towards the ends of the chromosome. At the population level, analysis of closely related strains (rDNA 16S 100% identical) confirmed this phenomenon by revealing early events occurring during diversification. The absence of natural transformation or transducing phages suggests that diversification results from conjugative transfer. Indeed, actinomycetes genomes revealed an important wealth in integrated and conjugative elements. We have also shown that genomic diversity contributes to the functioning of the *Streptomyces* population in the micro-habitat, promoting the production of common goods by a fraction of the population (Tidjani et al., 2019). This result is further supported by the demonstration by D. Rozen's team (Uni. Leiden) that within a colony, the presence of mutants with rearranged chromosomes influences the antibiotic production capacity of the entire population (Zhang et al., 2020). Altogether, these results show that the ability to rearrange its genome allows *Streptomyces* to diversify its genome content and could contribute to its adaptability to the soil, which is a naturally challenging ecosystem.

References

Tidjani et al., 2019. Massive Gene Flux Drives Genome Diversity between Sympatric Streptomyces Conspecifics. mBio. Sep 3;10(5):e01533-19.
Tidjani et al., 2020. Telomeric and sub-telomeric regions undergo rapid turnover within a Streptomyces population. Sci Rep. May 7;10(1):7720.
Zhang et al., 2020. Antibiotic production in Streptomyces is organized by a division of labor through terminal genomic differentiation. Sci Adv. Jan 15;6(3):eaay5781.

Dynamics of the compartmentalized *Streptomyces* chromosome during metabolic differentiation

Virginia Lioy[1,*], Jean-Noël Lorenzi[1], Soumaya Najah[1], Thibault Poinsignon[1], Hervé Leh[1], Corinne Saulnier[1], Bertrand Aigle[2], Sylvie Lautru[1], Annabelle Thibessard[2], Olivier Lespinet[1], Pierre Leblond[2], Yan Jaszczyszyn[1], Kevin Gorrichon[1], Nelle Varoquaux[3], Ivan Junier[3], Frédéric Boccard[1], Jean-Luc Pernodet[1], Stéphanie Bury-Moné[1]

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Streptomyces are among the most prolific bacterial producers of bioactive compounds, including antibiotics. In addition to having an unusual linear chromosome, their genome is segmented with the extremities enriched in specialized metabolite biosynthetic gene clusters. The molecular mechanisms that shape the structure and function of these compartmentalized genomes remain mostly unknown. By combining RNA sequencing and chromosome conformation capture, we explored the dynamics of the linear genome of *Streptomyces ambofaciens* during metabolic differentiation. We show that in exponential phase, the central region enriched in highly persistent genes is structured in several domains by active transcription. In the same conditions, the terminal ends form two large poorly structured and transcriptionally silent compartments rich in specialized metabolite biosynthetic gene clusters. Importantly, during stationary phase, metabolic differentiation is accompanied by a remodeling of chromosome architecture. Together, our results provide a link between genome evolution, gene expression and chromosome folding in *S. ambofaciens*.

On the Risks of Phylogeny-Based Strain Prioritization for Drug Discovery: *Streptomyces lunaelactis* as a Case Study

First name LAST NAME[1], First name LAST NAME [2] Name of the presenting author underlined

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Strain prioritization for drug discovery aims at excluding redundant strains of a collection in order to limit the repetitive identification of the same molecules. In this work, we wanted to estimate what can be unexploited in terms of the amount, diversity, and novelty of compounds if the search is focused on only one single representative strain of a species, taking *Streptomyces lunaelactis* as a model. For this purpose, we selected 18 *S. lunaelactis* strains taxonomically clustered with the archetype strain *S. lunaelactis* MM109^T. Genome mining of all *S. lunaelactis* isolated from the same cave revealed that 54% of the 42 biosynthetic gene clusters (BGCs) are strain specific, and five BGCs are not present in the reference strain MM109^T. In addition, even when a BGC is conserved in all strains such as the *bag/fev* cluster involved in bagremycin and ferroverdin production, the compounds produced highly differ between the strains and previously unreported compounds are not produced by the archetype MM109^T. Moreover, metabolomic pattern analysis uncovered important profile heterogeneity, confirming that identical BGC predisposition between two strains does not automatically imply chemical uniformity. In conclusion, trying to avoid strain redundancy based on phylogeny and genome mining information alone can compromise the discovery of new natural products and might prevent the exploitation of the best naturally engineered producers of specific molecules.

Genome dynamics of the *Streptomyces* genus

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Streptomyces possess a large linear chromosome (6-12 Mb) consisting of a conserved central region flanked by variable arms covering several megabases. In order to study the evolution of the chromosome across evolutionary times, a representative panel of *Streptomyces* strains and species (125) whose chromosomes are completely sequenced and assembled was selected. The pan-genome of the genus was modelized and shown to be open with a core-genome reaching 1,018 genes. The evolution of *Streptomyces* chromosome was analyzed by carrying out pairwise comparisons, and by monitoring indexes measuring the conservation of genes and their synteny along the chromosome. Using the phylogenetic depth offered by the chosen panel, it was possible to infer that the chromosomal arms evolved faster than the central region under the combined effect of rearrangements and the addition of new information from horizontal transfer.

Diversity and mobility of Integrative and Conjugative Elements in a population of *Streptomyces* from a rhizospheric micro-habitat

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Abstract

Streptomyces are ubiquitous soil bacteria, particularly in the rhizosphere, where they can promote plant growth and health. In a previous study [1], a comparative genomics study showed that a rhizospheric population of *Streptomyces* (isolated at the microscale) is composed of closely related individuals with significant genomic variability. Integrative and Conjugative Elements (ICEs) represent a significant part of this variability. Integrative and Conjugative Elements (ICE) present in *Streptomyces* belong to two families: those whose transfer depends on the assembly of a complex type IV secretion system (T4SS), and those more frequent in Actinobacteria depending of a single DNA FtsK/SpoIIIE-like translocation protein, the so-called AICEs for Actinobacteria ICEs. We hypothesize that ICE/AICE constitute the driver of genomic and functional diversity in *Streptomyces* thanks to the mobilization of chromosomal markers that accompany their own transfer.

We first aimed at characterizing the ICEs present in our *Streptomyces* population (11 isolates) and identified *in silico* 27 different ICEs including 26 AICEs. Those later ranged from 11,9 to 44,1 kilobases with some harboring adaptive genes. Each strain had an average of 5 conjugative elements but none were present in all strains suggesting a significant dynamic of these elements in our population. We further mapped the ICE transfers in the population among the 110 (11x10) possible conjugation pairs. The transfer of AICEs was observed for 13 of them, sometimes with a high frequency (100%). Finally, in order to estimate the intensity of gene fluxes associated to the ICE transfer (i.e. co-transfer of chromosomal markers), we selected couples of strains differing by their ICE contents and marked them at different chromosomal loci to select for the formation of recombinants. The fine analysis of the recombinant genome will be undertaken and will enable to estimate the extent of gene transfer within a natural population.

This study will provide a better understanding of the impact of gene transfer on the adaptation of microbial populations in the rhizosphere and on the role of the plant in intraspecific diversification mechanisms.

Keywords

Streptomyces, microbial population, conjugation, genetic diversity, rhizosphere.

[1] Tidjani A-R *et al.*, mBio 2019 ; 10: e01533-19

Charaterization of congocidine-inducible promoters

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The production of specialized metabolites by *Streptomyces* bacteria is usually temporally regulated and often begins with the development of the aerial mycelium. This regulation is complex and frequently involves both global and pathway-specific mechanisms. Our team studied the regulation of congocidine (netropsin) biosynthesis in *Streptomyces ambofaciens* ATCC 23877. We identified an atypical orphan response regulator, Cgc1, which activates the transcription of all *cgc* genes during metabolic differentiation. We also observed that exogenous congocidine can specifically induce the transcription of *cgc1* and the operonic resistance genes, *cgc20* and *cgc21*, encoding an ABC transporter. Remarkably, this induction, still observed in absence of Cgc1, does not lead to the expression of the other congocidine biosynthetic genes. Transcriptional fusions, using *gusA* as a reporter gene, were thus used to investigate the activity of the bidirectional *cgc1-cgc20* promoter region in heterologous models. In *Streptomyces lividans* TK23, P*cgc20* and P*cgc1* promoters were both induced by congocidine. This new induction system does not require heterologous expression of resistance genes, the latter having a negative impact on the level of induction in accordance with an efflux-based resistance mechanism. Experiments suggest that these newly characterized promoters are also active and inducible in *Escherichia coli*. Altogether, our results highlight the interest of investigating the specialized metabolite regulatory network to design new promoters for biotechnological applications using Actinobacteria.

Comparison of *Actinobacteria* communities according to *hsp*65 and *16S rRNA* genes in anthropogenic and pristine caves

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Background: Caves are isolated and nutritionally limited environments thus representing extreme conditions in which only few bacterial taxa are able to survive. Actinobacteria were found to be highly abundant in bacterial communities of many caves. However, most studies focus on actinobacterial isolates or study their community using *16S rRNA*, which is not sufficiently variable to distinguish related actinobacteria species. More precise identification of actinobacteria communities is necessary for searching novel or pathogenic members and understand their role in caves in relation to anthropogenic factor

Objectives: To determine actinobacterial community composition according to two gene markers from anthropogenic and pristine caves in France and from Lascaux cave walls also from stained and unstained zones. Identification of Actinobacteria members using *hsp*65 gene marker up to the species level.

Methods: We extracted DNA from four caves in France more or less affected by humans (Lascaux, Rouffignac, Reille, Mouflon), while from Lascaux cave from from stained and unstained zones of different areas (SAS-1, Passage - banks, Passage - inclined planes, Apse, Diaclase) and using specific primers for *16S rRNA* gene (for bacteria) and *hsp*65 (for Actinobacteria) we sequenced the related genes by Illumina MiSeq. The sequences were processed by MOTHUR and distance matrices were compared to assess the community composition differences between the caves according to the both gene markers. Database of *hsp*65 genes was desinged to classify Actinobacteria members into the species.

Results: Our results showed that both gene markers specifically describe differences of the Actinobacteria communities between the caves. According to both markers, Actinobacteria community structure differed among visited (rouffignac), previously visited but already closed (Lascaux) and pristine (Reille, Mouflon) caves. According to *hsp*65 gene marker, potential pathogenic actinobacterial members from *Nocardia* genera were identified in anthropized caves and domination of *Streptomyces* genera were found in stained zones of Lascaux cave. Our results confirmed the potential of *hsp*65 gene marker for monitoring of uncultured Actinobacteria strains in environment.

Treboniaceae, a novel actinobacterial family from acidic environments

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A novel group of previously undescribed actinobacteria was observed to dominate several locations in Czech Republic in acid soils. The 16S rRNA gene phylogeny of the environmental clones indicated not only a vast diversity, but also the separate position of this clade at the possible level of a new order [1]. The closest strains were isolated during a wide studies of uncultured bacteria but have not been described [2,3].

We isolated and described *Trebonia kvetii gen. nov., sp. nov*. [4], a slowly-growing acidiphilic actinobacterium from the waterlogged soil, and its place within the soil bacterial community. In the poster we would present *Treboniaceae* family and some aspects of the cultivation of “uncultured” bacteria.

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Identification by LC-MS/MS of metabolites from bioconversion of chlorogenic acid, a dietary phenolic compound, by gut bacterial species

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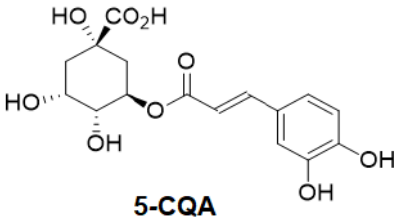
Polyphenols, phytochemicals derived from natural sources, have drawn increasing attention from the scientific community in their ability to lower oxidative stress involved in many pathologies and on their positive effects in modulation of redox cellular signalling pathways [1]. However, since they are characterized by a low bioavailability and given the crucial role of microbiota to generate bioactive and bioavailable metabolites [2], more insights are focused nowadays on their gut-derived metabolites as promising approach to prevent and attenuate neurodegenerative diseases [3].



5-CQA

5-Caffeoylquinic acid (5-CQA) known as chlorogenic acid, a dietary phenolic compound, has already shown neuroprotective properties [4] and some of its microbial gut-derived metabolites are already known [5]. In this work, the bioconversion of 5-CQA has been studied by intestinal bacterial species; *Bifidobacterium longum (Actinobacterium), Bacteroides fragilis (Bacteroidetes)* and *Lactobacillus reuteri (Firmicutes)* in mono and co-cultures. The extracts after incubation of bacteria with 5-CQA were analyzed by LC-MS/MS and particular attention has been be paid on oxidized compounds which could arise from redox pathways. In addition, an electrochemical strategy was adopted to generate oxidized compounds of chlorogenic acid in phosphate buffer in order to characterize and compare their mass profiles. A molecular networking through GNPS platform [6] was also employed to identify the biotransformed metabolites.

L. reuteri has shown capacity to biotransform 5-CQA notably into caffeic acid (CA) and its oxidized derivative quinone and into 3-Hydroxbenzoic acid (HBA), contrary to *B. fragilis* and *B. longum* which didn't show any ability. Nevertheless, first experiments in co-cultures exhibited an interesting pattern in bioconversion of 5-CQA.

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[4] Heitman E *et al.,* Nutritional Neuroscience. 2017; 20: 32–39.
[5] Mortelé O *et al.*, J. Pharm. Biomed. Anal. 2019; 175: 112768.
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What can Mass Spectrometry reveal about specialized metabolites of bacteria?

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Among modern analytical tools, Mass Spectrometry (MS) offers a large variety of applications, including proteomics and metabolomics, in the field of bacteria research. In particular, due to high mass resolution and precision measurements, together with tandem MS for structural characterization, MS emerged as a gold standard for the study of specialized metabolites from bacteria.

Three main developments performed through collaborations at ICSN will be introduced here:

- MS classification of bacterial strain by MALDI-TOF fingerprint (biotyping): after short time of cultivation, lipid or protein extract can be performed from a single Petri dish in order to acquire a MS fingerprint and compare them to database or from each other. This classification is efficient and fast to classify bacteria showing same phenotypes before genomic identification [1].

- Molecular network: dereplication is one of the crucial step to identify known specialized metabolites and to focus research tasks on unknown ones. LC-MS/MS is highly efficient to separate, detect and elucidate partial structures of specialized metabolites. In order to decipher the large amounts of generated data, molecular network is becoming unavoidable in this field. Several examples related to the use of MetGem Software developed at ICSN will be introduced [1,2].

- Mass Spectrometry Imaging: MS imaging allows determining the distribution of metabolites on biological surfaces including transfers of Petri dishes at a resolution of fen tens of micrometers. In particular, accumulated *versus* released compounds can be imaged and competition experiments can be surveyed at different time scales [3].

All these techniques are now available for the bacteria community leading to emerging interdisciplinary projects.

[1] Barthelemy M *et al.*, Sci. Rep. in press, DOI: 10.1038/s41598-020-74852-w
[2] Olivon *et al,* Anal. Chem. ; 90(23): 13900-13908.
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A Single Biosynthetic Gene Cluster Is Responsible for the Production of Bagremycin Antibiotics and Ferroverdin Iron Chelators

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Biosynthetic gene clusters (BGCs) are organized groups of genes involved in the production of specialized metabolites. Typically, one BGC is responsible for the production of one or several similar compounds with bioactivities that usually only vary in terms of strength and/or specificity. Here we show that the previously described ferroverdins and bagremycins, which are families of metabolites with different bioactivities, are produced from the same BGC, whereby the fate of the biosynthetic pathway depends on iron availability. Under conditions of iron depletion, the monomeric bagremycins are formed, representing amino-aromatic antibiotics resulting from the condensation of 3-amino-4-hydroxybenzoic acid with pvinylphenol. Conversely, when iron is abundantly available, the biosynthetic pathway additionally produces a molecule based on p-vinylphenyl-3-nitroso-4-hydroxybenzoate, which complexes iron to form the trimeric ferroverdins that have anticholesterol activity. Thus, our work shows a unique exception to the concept that BGCs should only produce a single family of molecules with one type of bioactivity and that in fact different bioactive molecules may be produced depending on the environmental conditions [1].

[1] Martinet L *et al.*, mBio Vol 10-2019 ; 4 : 1-34.

A highly promiscous cytochrome P450 adds nitrogenous bases to tyrosine-containing cyclodipeptides

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[#] Equal contribution

Mining the genome of *Streptomyces flavotricini* TN58 allowed the identification of a gene cluster encoding a cyclodipeptide synthase and cytochrome P450 and susceptible to direct the biosynthesis of diketopiperazines. Expression of these two genes in *Streptomyces* strains accompanied or not of precursor feeding led to the production of 26 compounds forming a new family of diketopiperazines: the sfaximycins. Their synthesis involves a cytochrome P450 tailoring enzyme that mainly catalyzes the addition of nitrogenous bases (guanine, xanthine, hypoxanthine, adenine, and cytosine) on tyrosine side-chains of cyclodipeptides. Coupling of cyclodipeptides with nitrogenous bases through an ether bond is reported here for the first time. The cytochrome P450 tailoring enzyme described in this work is very promiscuous and may constitute a useful tool to expand the chemical diversity of CDPs derivatives and give rise to new interesting biomolecules.

Engineering of a Polyketide Synthase Leads to 'Low-fat' Stambomycins

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Bacterial polyketide secondary metabolites exhibit a wide range of useful biological properties, including antibiotic, antifungal, anti-tumor and immunosuppressive properties. The stambomycins A–F are a small family of compounds recently discovered from *Streptomyces ambofaciens* ATCC23877 by genome mining, which exhibit promising anti-cancer activity [1, 2]. However, given their enormous size (at 51-members, the macrolactone core of the stambomycins is among the largest of all known polyketides), there is substantial interest in accessing smaller derivatives that might retain the bioactivity of the parental structures.[3]

In this project, we aimed to use synthetic biology to modify the stambomycin biosynthetic pathway towards the generation of such 'mini-stambomycins'. The pathway notably contains a modular polyketide synthase (PKS) which generates the stambomycin macrolactone, and which comprises 9 distinct polypeptide subunits, housing in total 25 'modules' of functional domains. To access the desired smaller derivatives, we have leveraged our current understanding of protein-protein interactions in modular PKS systems to manipulate several key contacts in the stambomycin PKS which mediate both chain translocation and elongation. Characterization of the multiple, resulting 'low-fat' analogues is currently in progress.

Key words: polyketide synthase, genetic engineering, structural simplification

References:

1. Laureti, L., *et al.* (2011) Identification of a bioactive 51-membered macrolide complex by activation of a silent polyketide synthase in *Streptomyces ambofaciens*. *Proc. Natl. Acad. Sci. USA* **108**, 6258–6263.

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3. Wang, S. *et al.* (2019) Structural simplification of natural products. *Chem. Rev.* **119**, 4180–4220.

Refactoring of the congocidine biosynthetic gene cluster, a first step towards combinatorial biosynthesis of pyrrolamide non-ribosomal peptides

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[2]Molecular Microbiology of Actinomycetes, Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, University Paris-Sud, University Paris-Saclay, Orsay, France.

Non-ribosomal peptides (NRP), used in medicine in particular as antibiotics (vancomycin, daptomycin) or immunosuppressants (cyclosporins), are synthetized by non-ribosomal peptide synthetases (NRPS). These enzymes are modular, thus adapted to synthetic biology approaches based on module exchanges, such as combinatorial biosynthesis, allowing the synthesis of new NRP. Combinatorial biosynthesis consists in combining biosynthetic genes coming from a group of biosynthetic genes of diverse specialized metabolites in order to generate analogs. Studies have proved the feasibility of this approach using the NRPS, but the yields obtained vary greatly, and reasons of these variations are not clearly understood [1].

In this study, we aim at understanding the factors impeding NRPS-based combinatorial approaches using the pyrrolamide family as a model. NRPS of the pyrrolamide family are non-canonical NRPSs made of stand-alone modules and domains [2], which makes them particularly amenable to genetic and biochemical manipulations. Furthermore, there are examples of natural combinatorial biosynthesis among the pyrrolamide family [3]. As first steps toward our goal, we report here (i) the construction of a set of standardized vectors for *Streptomyces* synthetic biology applications (Figure 1), (ii) the construction of gene cassettes for pyrrolamide combinatorial biosynthesis and (iii) the refactoring of the congocidine gene cluster as a proof of concept.

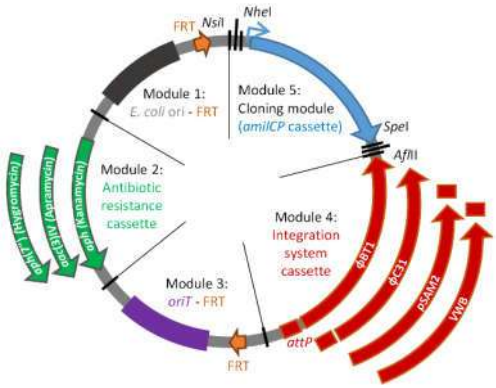

Figure 1: Schematic representation of the set of modular and integrative vectors (available on request at Addgene and BCCM genecorner)

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Symbiosis between *Frankia* and actinorhizal plants: from ecosystem to molecules of interaction

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To cope with the rarity of nitrogenous compounds, certain plants develop highly sophisticated systems to interact with nitrogen-fixing bacteria. Through a complex cross-talk with those diazobacteria, these plants can develop specialized organs, called nodules that house the bacteria. Nodulation involves two types of diazotrophic bacteria: rhizobia that associate symbiotically with legumes (Fabales) and with one nonlegume genus, Parasponia (Rosales); and filamentous actinobacteria of the genus *Frankia*, which can remarkably induce nodulation on a very diverse group of plants from the Fagales, Rosales, and Cucurbitales collectively called actinorhizal plants. This actinobacteria is able to interact with the plant, to penetrate the root tissues and then to fix atmospheric nitrogen and transform it into nutrients that can be assimilated by the plant. Most of actinorhizal plants are perennial woody shrubs or trees distributed all around the word. They play pioneer roles by colonizing nitrogen-poor sites before ecological transitions. Most strikingly, actinorhizal symbioses fix most (over 15%) of the biologically available nitrogen into ecosystems, making this symbiosis a key player in the nitrogen cycle. This interaction has an important impact on biodiversity and nitrogen cycle in the environment. The success of this interaction depends on the recognition of the right partner by the plant from a vast diversity of surrounding bacteria abundantly present in the rhizosphere. Understanding the molecular mechanisms by which plants recognize, discriminate 'friends' from 'foes' for further drive an efficient cohabitation, is a considerable challenge.

Genome mining of *Streptomyces violaceusniger* AgN23 highlights its high potential as plant stimulant and biological control agent

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The Shared Laboratory LRSV/ De Sangosse looks for microbes and natural products for crop growth stimulation and protection from disease. We selected AgN23, a *Streptomyces* strain isolated from wine grape roots, for its capacity to stimulate the plant immune system, thereby protecting plants against fungal pathogens [1]. We sequenced its genome with Pacbio technology corrected by Illumina Hiseq. We could assemble a 10.9 Mb chromosome-scale contig with a depth of 77X and no plasmid detected. Phenotypic observation coupled with phylogenomic analyses attributed AgN23 to the *S. violaceunsiger* species. AgN23 genome has an Average Nucleotide Identity (ANI) higher than 90% with strains found all around the globe in diverse soils, often as plant rhizosphere-colonisers (e.g. *S. violaceusniger* Tü 4113, *S. hygroscopicus* XM201 or *S. bingchenggensis* BCW-1). It suggests that these bacteria could associate with plants. Genome mining allowed us to identify 45 regions containing specialised metabolites biosynthetic gene clusters (SMBGCs). We found that 31 SMBGCs are strongly conserved among eight phylogenetically related strains, revealing the core genome of *Streptomyces violaceusniger* species. We are currently functionally investigating three candidate SMBGCs for the biosynthesis of plant defence elicitors, one of them being conserved by the closest strains. Thus, bioactivity of these strains toward the plant may be conserved across these isolates. This work therefore emphasises the potential of the *Streptomyces violaceunsiger* clade for housing interesting strains for the development of plant protection products such as our strain AgN23.

Key words: *Streptomyces violaceusniger*; plant stimulant; biological control; plant defence elicitor; genomics; phylogenomics

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Roots and rhizosphere colonization by a soil borne Streptomyces strain stimulates roots defence and development and promotes microbiota diversity

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AgN23 is a *Streptomyces* strain belonging to the *violaceusniger* clade, which was firstly isolated from grapevine rhizosphere. AgN23 colonises plant surface tissues, where it shows elicitation of plant defense along with protection against fungal pathogens. Here, we investigated the interaction of AgN23 with *Arabidopsis thaliana* roots. Using mCherry and GFP labelled AgN23 strains, we showed that the plant supports extensive AgN23 growth on root surface (rhizoplane) but without signs of endophytic development. In an *in vitro* system, we observed that the bacteria modifies root architecture, increasing lateral roots formation and limiting the elongation of the primary root, and promotes protection against a root pathogenic oomycete (*Phytophthoracapsici*). To identify molecular mechanisms underlying the activity of AgN23 on plant development and interaction with pathogens, a global metabolomics study of *Arabidopsis* plants inoculated with AgN23 was performed. This analysis showed that the bacteria stimulates the biosynthesis of plant secondary metabolites involved in plant defense such as flavonoids and indole glucosinolates, notably camalexin, a major antimicrobial compound of *Arabidopsis*. Finally, we studied the behavior of AgN23 upon inoculation of potting soil. Tracking AgN23 DNA in the soil revealed that the strain accumulates more in the rhizospheric soil rather than in bulk or unplanted soil. Analysis of rhizosphere microbiota showed a clear re-structuration of the bacterial communities in presence of AgN23, with a significantly enhanced alpha diversity. Together, our results suggest that a single rhizospheric *Streptomyces* strain can promote root protection and development through the stimulation of plant immunity and by increasing the root microbiota diversity, illustrating the tremendous impact of this ubiquitous bacteria genus in plant health and development.

Rôle d'un peptide antimicrobien de la famille des « lipid transfer protein » lors de la symbiose actinorhizienne

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Les plantes actinorhiziennes telles que l'Aulne glutineux sont capables de coloniser les biotopes pauvres en azote grâce à leurs capacités à établir des symbioses avec des actinobactéries filamenteuses fixatrices d'azote du genre *Frankia* [1]. La reconnaissance entre les deux partenaires initie un programme symbiotique au niveau des racines aboutissant à la formation d'un nouvel organe, le nodule, dédié à l'accueil de la bactérie et aux échanges trophiques. Des études transcriptomiques menées sur l'Aulne glutineux ont permis d'identifier les gènes différentiellement exprimés lors des étapes précoces de la symbiose (2 jours post-infection) et dans le nodule (21 jours post-infection [2, 3]). Lors des étapes précoces de la symbiose 75% des gènes surexprimés codent des peptides sécrétés. Parmi ceux-ci, un gène codant une « lipid transfer protein », AgLTP24, qualifiée de peptide antimicrobien, est le plus surexprimé dans les étapes précoces mais aussi dans la nodosité. Cela suggère un rôle important d'AgLTP24 de la mise en place et dans le maintien de la symbiose. Ces travaux ont pour objectifs de localiser AgLTP24 dans les tissus végétaux à ces différents stades de la symbiose et de déterminer ses effets physiologiques sur le symbiote bactérien, Frankia, afin d'identifier de possibles fonctions biologiques.

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Biocontrôle de *Rhizoctonia solani,* agent causal de la fonte des semis de la tomate, par des actinobactéries endophytes du genre *Streptomyces* spp. et leur effet PGPB.

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Résumé

L'objectif de ce travail porte sur l'étude du pouvoir des actinobactéries endophytes isolées de quelques espèces sahariennes dans le biocontrôle de *Rhizoctonia solani*, agent causal de la fonte des semis de la tomate. Leurs effets sur la promotion de la croissance des plantules (PGPB) ont été également étudiés.

Quarante et une souches d'actinobactéries ont été isolées à partir des racines de 13 espèces sahariennes. L'étude morphologique et l'isomérie de l'acide diaminopimélique ont permis de rattacher 36 isolats au genre *Streptomyces* et de considérer le reste comme non-*Streptomyces*.

Tous les isolats ont été étudiés pour leurs capacités à réduire l'incidence de la maladie en culture *in vitro*. La bactérisation des semences de tomate par les spores de 15 isolats d'actinobactéries a permis de réduire l'IM à des valeurs inférieures à celle du Sérénade®. Ainsi, les 15 isolats performants ont été retenus pour un deuxième screening de biocontrôle *in vivo* en sol stérilisé et en sol non stérilisé. L'effet PGPB, par la mesure des poids frais et sec et la longueur des plantules et des racines, a été également déterminé. Seuls 9 isolats ont gardé leurs potentiels de biocontrôle *in vivo*. De plus, l'isolat *Streptomyces* sp. ZL2, isolé à partir de *Ziziphus lotus*, s'est montré le plus efficace. Par ailleurs, l'effet PGPB sur les plantules bio-protégées était variable selon les traitements. En vue d'une meilleure visualisation des résultats, une analyse en composante principale (ACP) a été effectuée. Elle a permis de sélectionner et de classer, respectivement, les isolats *Streptomyces* spp. ZL2 et CA2 selon leur efficacité de biocontrôle et d'effet PGPB. La taxonomie moléculaire a permis de rapprocher l'isolat *Streptomyces* sp. ZL2 à *Streptomyces caeruleatus* avec 99,4% de similarité et l'isolat *Streptomyces* sp. CA2 à *Streptomyces lydicus* aves 100% de similarité.

Les résultats obtenus ouvrent des perspectives prometteuses pour les isolats ZL2 et CA2 dans le biocontrôle de la fonte des semis de la tomate.

Mots clés: actinobactéries endophytes; *Streptomyces* spp; biocontrôle; *Rhizoctonia solani*; tomate.

Preparation of biofungicides based on *Nocardiopsis dassonvillei* strain MB22 spores for biocontrol of *Bipolaris sorokiniana* in durum wheat plants

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Abstract

Nocardiopsis dassonvillei strain MB22, isolated from an Algerian Saharan soil showed antifungal activities against 5 major wheat fungal pathogens. The strain was found positive for production of fungal cell wall degrading enzymes such as chitinases, lipases, proteases, and also produced sidérophores.

The strain MB22 showed positive plant growth promoting traits. It produced up to 95 µg/ml of IAA and solubilized a significant amount of inorganic phosphate. The strain produced ammonia and hydrogen cyanide in vitro.

Durum wheat seeds bacterized with spores of MB22showed the highest biocontrol activity with a disease severity (DSI) caused by *Bipolaris sorokiniana* index lower than non-treated seeds. No significant difference ($p < 0.05$) was observed between the DSI of plants obtained from the strainMB22 and those derived from seeds treated with the chemical fungicide Dividend®. In pot trial experiments, MB22-treated durum wheat plants showed significant growth improvement. The strain was formulated in talc powder and sodium alginate beads and the shelf-lives were monitored. Talcum formulation showed higher cell-count than sodium alginate beads even after 12 months of storage, and optimum condition for storage of the powder formulation were found to be at 4°C. Talcum powder formulation based on spores of MB22 enhanced the plant resistance to *B. sorokiniana* root rot and promoted the growth of durum wheat seedlings.

Keywords

Durum wheat, Biocontrol, Plant growth promoting activities, Biofungicides, *Nocardiopsis dassonvillei* MB22.

Mycoloyltransferases: a multi-task family of enzymes involved in cell envelope biogenesis.

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Corynebacteriales are a phylogenetic order of actinobacteria that includes numerous human pathogens such as Mycobacterium tuberculosis, but also the agents of leprosy, diphtheria, as well as emergent pathogens causing opportunistic diseases. All these genera share, among other characteristics, the ability to synthetize mycolic acids which are unique and major components of a non-conventional outer membrane (mycomembrane). These mycolic acids result from the condensation of two acyl chains by a cytoplasmic polyketide synthase (Pks13). After reduction and esterification on trehalose, they are further exported to the cell wall where they are ultimately loaded on their various final acceptors by a family of enzymes called Mycoloyltransferases (Myts). The catalytic activities of Myts were carefully characterized in vitro using various analogs of mycolatedonnors and -acceptors but their precise role in vivo is still lacking or incomplete. In our laboratory, we use the model organism Corynebacterium glutamicum, in which we identified 5 mycoloyltransferases isoforms. In this presentation, I will mostly focus on our recent findings concerning MytA and MytC contributions to the mycomembrane biogenesissuggesting that Myts functions are far less redundant than previously expected and play multiple important functions during the complex biogenesis of the cell envelope.

Waking-up silent bacterial genes for natural product discovery

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Mining bacterial genomes has revealed a vast number of gene clusters proposed to direct the biosynthesis of novel specialized natural products. However, many of these gene clusters remain silent, or are poorly expressed, in laboratory growth conditions.

Our research group is particularly interested in investigating the molecular mechanisms by which pathway-specific transcriptional regulators control the expression of silent and cryptic biosynthetic gene clusters [1, 2].

By exploiting our structural and functional understanding of specific classes of transcriptional regulators, we have successfully uncovered novel families of natural products and, subsequently, novel biocatalysts [3,4].

This presentation will first introduce some of the fundamental work we have carried out before discussing the specific strategies we have used to access the metabolic products of silent biosynthetic gene clusters.

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In silico driven natural product discovery by unveiling the binding sites of uncharacterized transcription factors

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Advances in genome sequencing have shown that the biosynthetic potential of *Streptomyces* and other antibiotic-producing Actinobacteria has been largely underestimated, as suggested by the surprisingly large number and diversity of biosynthetic gene clusters (BGCs) in their genomes. However, many of the BGCs are not expressed at sufficiently high levels to allow detection of the compounds they produce. Interestingly, the genomes of streptomycetes contain an unusually high percentage (>10%) of regulatory genes, reflecting their ability to quickly adapt to changing environmental conditions [1]. Specialised metabolism is often considered to be an essential part of their adaptive response, and can be a reaction to stress or a means of communication in inter- or intra-species interactions [2]. Cracking the regulatory code for these BGCs, in other words, finding the transcription factors (TF) and the associated *cis*-regulatory elements (CRE) regulating BGCs, will enable us to find new ways to activate the production of bioactive compounds [3]. For the vast majority of the TFs, the cognate CRE is currently unknown. Here, I will present a method that enriches the database of known TF-CRE couples. This enables us to perform genus-wide regulon predictions, and makes it possible to link certain regulators to both primary and secondary metabolism. This method is based on the fact that orthologous TFs bind a conserved regulatory motif on the DNA, and that in some families, like the LacI family, most genes are autoregulated [3,4,5]. This allows rapid identification of candidate binding sites. We were able to predict regulatory motifs for over 50 yet unstudied LacI TFs. Using these predictions, a new catabolic pathway for mannose/mannan-derived sugar utilization was uncovered, which is controlled by ManR. This information was then exploited to predict links between primary and secondary metabolism.

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From elicitor perception to nutrient acquisition: insights into the specific lifestyle of *Streptomyces scabies*

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Streptomyces scabies is often considered as the odd one out among the *Streptomyces* genus due to its generally accepted pathogenic lifestyle. This species, along with a few related streptomycetes, is responsible for the common scab disease affecting tuber crops with potato (*Solanum tuberosum*) cultures being the most widely impacted [1]. Despite the recent elucidation of the signaling cascade triggering the virulent behavior, many aspects associated with the lifestyle of *S. scabies* require further investigation. A genome mining approach allowed us to uncover a huge potential for secondary metabolites production. Despite decades of research on this model species, only a few bioactive compounds – mostly plant-associated – have been described and associated to their respective biosynthetic gene clusters (BGCs). The majority of the clusters are thus considered as 'cryptic' and remain to be investigated. The response of the core biosynthetic genes to the elicitors of the pathogenic lifestyle – cellobiose and cellotriose – was evaluated through a transcriptomic approach. The data obtained highlighted the response of several clusters – including cryptic ones – to the elicitors, potentially indicating novel BGCs involved in the plant-associated lifestyle.

At the other end of the signaling cascade, nutrient acquisition remains the main target for a pathogen under host infection. Because of the use of starch as storage carbohydrate by tuber crops, *S. scabies* is expected to consume this polysaccharide and its derivatives to complete its lifecycle. Surprisingly, the disaccharide maltose is a poor carbon source for the growth of *S. scabies*. This indicates, as in other streptomycetes [2,3], that the regulator MalR exerts a strong repression on the genes involved in maltose consumption. The study of *malR*/MalR in *S. scabies* confirmed this function in this species and showed that it is a target for frequent spontaneous mutations conferring increased maltose utilization capacities. Further investigation in the role of MalR in nutrient acquisition and by extent pathogenicity holds a great potential for the understanding of the common scab causal agent and its lifestyle.

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Neuron-*Mycobacterium* interactions

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ABSTRACT

Infection with *Mycobacterium ulcerans* or Buruli ulcer has become the third most common mycobacteriosis after tuberculosis and leprosy. This infection causes large ulcers, which despite their severity are painless, suggesting that the bacillus is acting on the peripheral nervous system. In patients, the lack of sensitivity does not result from a destruction of the nerves since patients regain sensitivity upon treatment with antibiotics. In view of this observation, we hypothesized that the toxin secreted by *M. ulcerans* (mycolactone) may have analgesic properties by disrupting the passage of nerve impulses. Indeed, we showed that mycolactone activates the angiotensin 2 receptor 2 (AT2R) leading to the opening of K+ channels (TRAAK) and cell hyperpolarization. Recently, using different in vivo models, we demonstrated the analgesic potential of mycolactone. Our results open new perspectives for the development of bioinspired painkiller candidates.

KEYWORDS

Mycolactone, *Mycobacterium ulcerans*, analgesia, angiotensine 2 receptor 2, TRAAK.

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