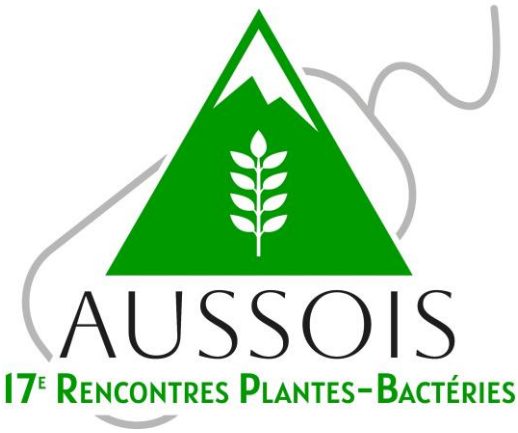
Abstract Booklet



January 13-17, 2025

We are pleased to welcome you at the 17th Plants Bacteria Meeting and thank you all for your much-appreciated contributions.

--

The Organizing Committee (Angers, IRHS)

Alba Noël, Alexandre Degrave, Armelle Darrasse, Marie Simonin, Marie-Agnès Jacques, Marion Barbier, Marion Fischer-Le Saux, Nicolas Chen, Perrine Portier & Sophie Cesbron.

Elodie Lemmens is acknowledged for her help for the preparation of this book. The picture on the cover was designed with ChatGPT's help.

Guest speakers

- **Delphine Capela,** LIPME, INRAE Occitanie Toulouse, France
- **Guilaume Chesneau,** Max Planck Institute for Plant Breeding Research, Köln, Germany
- **Yael Helman**, Hebrew University of Jerusalem, Rohovot
- **Samuel Jacquiod**, Université de Bourgogne, Dijon, France
- **Olaya Rendules,** Centre de Biologie Integrative, Toulouse
- **Adam Shikora,** Julius Kühn Institute (JKI), Braunschweig, Germany
- **Edward Topp,** Agroécologie, INRAE Bourgogne Franche Comté - Dijon, France
- **Laure Weisskopf,** Fribourg University, Switzerland

Scientific Committee

- **Wafa Achouak**, DR CNRS, Cadarache
- **Benoit Alunni**, DR INRAE, IJPB, Versailles
- **Marie-Anne Barny**, DR INRAE, Paris
- **Odile Berge**, CR INRAE, Avignon
- **Pierre Czernic**, Prof Montpellier University
- **Axel de Zélicourt**, MCF IPS2 - Université Paris Saclay
- **Pierre Frendo**, Prof Université Nice Sophia Antipolis, Nice
- **Lionel Gagnevin**, CR CIRAD, Montpellier
- **Alice Guidot**, CR INRAE, Toulouse
- **Florence Hommais**, Prof Lyon 1 University
- **Mathilde Hutin**, CR IRD, Montpellier
- **Frédéric Labbé,** CR CIRAD, La Réunion
- **Barbara Pivato,** CR INRAE, Dijon
- **Marie Simonin**, CR INRAE, Angers
- **Corinne Vacher,** DR INRAE, Bordeaux
- **Florence Wisniewski-Dyé**, Prof Lyon 1 University

Organizing Committee

- **Alba Noël**, MCF Université Angers, IRHS, Angers
- **Alexandre Degrave**, MCF Institut Agro Rennes-Angers, IRHS, Angers
- **Armelle Darrasse**, IR INRAE, IRHS, Angers
- **Marie Simonin**, CR INRAE, IRHS, Angers
- **Marie-Agnès Jacques**, DR INRAE, IRHS, Angers
- **Marion Barbier**, TR INRAE, IRHS, Angers
- **Marion Fischer-Le Saux**, CR INRAE, IRHS, Angers
- **Nicolas Chen**, MCF Institut Agro Rennes-Angers, IRHS, Angers
- **Perrine Portier**, IR INRAE, IRHS, Angers
- **Sophie Cesbron,** IE INRAE, IRHS, Angers

Sponsorship

The organizers warmly thank the following partners for their financial support of the 17th Plants Bacteria Meeting. Further information about these partners is available by clicking here

By alphabetical order:

- ✓ **Agro Innovation International – Groupe Roullier**
- ✓ **FRAIB - CNRS**
- ✓ **Gold Standard Diagnostics Millidrop**
- ✓ **INRAE SPE department**
- ✓ **Limagrain**
- ✓ **SFP (Société Française de Phytopathologie)**
- ✓ **Structure Fédérative de Recherche QuaSav - Qualité et Santé du végétal**
- ✓ **Temp Control**
- ✓ **Vegepolys Valley**

Academic sponsors

Private sponsors

Table of contents

Conference program

Monday, January 13th

17:00 19:00 REGISTRATIONS

19:15 20:00 Welcome drink

20:00 21:00 DINER

21:10 21:20 Opening of the 17èmes Rencontres Plantes Bactéries

21:20 22:00 Introductive SESSION

Moderators: Marie Simonin, Benoît Alunni

21:20 22:00 **Invited speaker Olaya Rendueles-Garcia**
The bacterial capsule as a key driver of microbial evolution

Tuesday, January 14th

08:45 11:50 SESSION Molecular dialog during plant-bacteria interactions: from symbiosis to pathogenicity

Moderators : Adam Schikora, Axel de Zélicourt

08:45 **Invited speaker Adam Schikora** Specific and general factors modulating plant response to bacterial N-acyl homoserine lactones

09:25 **Alicia Camuel** New insights into the T3SS-triggered nodulation between *Bradyrhizobium* and *Aeschynomene*

09:40 **Corinne Audran** *Xanthomonas campestris* pv. *campestris* Tal12a contribution to black rot disease and prediction of its host targets in cauliflower

09:55 **Laura Agert** Function and adaptation of symbiotic receptors that activate the Nod-independent symbiosis in the tropical legume *Aeschynomene evenia*

10:10 **Axelle Frantz** Characterization of the role of apple agglutinins in resistance against fire blight

10:25 BREAK

10:50 **Liam Lebeau** Mechanisms by which plant and synthetic small non-coding RNAs direct gene silencing in bacteria

11:05 **Marvin Navarro** Characterization of an adaptive mutation in a Type 6 effector that improves the proliferation of *Ralstonia solanacearum* in *Mimosa pudica* nodules

11:20 **Clara Blonde** The role of anaerobic respiration of carbon sources in the adaptation and survival of *Dickeya dadantii*

11:35 **Roza Mohammedi** Role of the CckA-ChpT-DivL complex in the phosphorylation of the master regulator CtrA during the cell cycle and nitrogen-fixing symbiosis in *Sinorhizobium meliloti*

11:50 **MY POSTER IN 60 SECONDS** odd numbers/numéros impairs posters n°1 to 49

12:30 13:30 LUNCH

13:30 17:00 FREE or ROUND TABLE DISCUSSION (according to weather condition)

17:00 **Alex Lheureux** MilliDrop: Unlocking Insights into Plant-Bacteria Interactions

17:15 19:25 SESSION Structure, function and engineering of the microbiome

Moderators: Samuel Jacquiod, Corinne Vacher

17:15 **Invited speaker** **Samuel Jacquiod** Artificial selection of rhizosphere microbiota altering plant phenotype: treating microbial communities as units of selection

17:55 **Logan Suteau** Multi-Kingdom Synthetic Communities Modulate Seedling Microbiota and Reveal Key Bacteria-Fungi Interactions in *Brassica napus*

18:10 **Amélie Caddéo** Population dynamics modelling of synthetic communities isolated from seed bean microbiota and their impact on pathogen *Xanthomonas citri* pv. *fuscans*

18:25 **Paola Fournier** Revealing microbial consortia that interfere with grapevine downy mildew through microbiome epidemiology

18:40 **Léa Jobert** Cry for help: A differential rice root-associated microbiome response to foliar infection by two pathogenic fungi

18:55 **Louna Colaert-Sentenac** Transient Seed Microbes, Long-Term Impacts: Seed Microbiota Affect Seedling Phenotype and Microbiome Assembly

19:10 **Barbara Pivato** Plant-microbe and plant-plant interactions favouring iron content in crop plants

19:25 **MY POSTER IN 60 SECONDS** odd numbers/numéro impairs posters n°51 to 59

19:45 20:45 DINER

21:00 **SESSION ODD NUMBER / CHIFFRE IMPAIR POSTERS**

Wednesday, January 15th

08:45 11:40 SESSION Physiology of plant-bacteria interactions: regulation, epigenetics, metabolites

Moderators: Yael Helman, Mathilde Hutin

08:45 **Invited speaker Yael Helman** For better or worse - the effect of interspecies microbial interactions on disease severity in plants

09:25 **Nicolas Burkhardt** Reduction of basal levels of (p)ppGpp in experimentally evolved *Ralstonia solanacearum* as a means of adaptation to plant xylem and legume symbiosis

09:40 **Tifaine Folletti et Pascal Ratet** Characterization of the pathogenic interaction between an atypical endophytic bacteria *Ensifer adhaerens* T4 and the legume *Medicago truncatula*

09:55 **Margaux Cheminat** Molecular and physiological consequences of the *Streptomyces* sp. GPA1 - Barley relationship

10:10 **Quentin Dubois** Unravelling the mechanisms of *ArcZ* sRNA in the virulence control of *Dickeya solani*

10:25 BREAK

10:55 **Gabriella Houdinet** Investigating leaf symbiosis: how do plants cope without their hereditary bacteria ?

11:10 **Ibrahim Keita** Role of the long non-coding RNA *SYNC1* in transcriptional reprogramming during nodule development

11:25 **Sara Moutacharrif** Identification of a bifunctional RNA involved in the virulence of *Dickeya dadantii*

11:40 **MY POSTER IN 60 SECONDS** Even numbers / numéros pairs posters n°2 to 48

12:30 13:30 LUNCH

13:30 17:00 FREE or ROUND TABLE DISCUSSION (according to weather condition)

17:00 19:10 SESSION Evolution, phylogeny, plant and bacterial genomics

Moderators: Delphine Capela, Lionel Gagnevin

17:00 **Invited speaker** **Delphine Capela** Unveiling the evolution of legume symbionts: beyond essential symbiotic gene acquisition

17:40 **Noé Arroyo-Velez** Analysis of the diversity of prophages in *Xylella fastidiosa*

17:55 **Frédéric Labbé** A Type 5 integrated prophage of "*Candidatus* Liberibacter asiaticus", the destructive bacterial pathogens of *Citrus* Huanglongbing

18:10 **Chloé Peduzzi** Evolutionary replacement of T4SS by T6SS for antibacterial killing activity in *Xanthomonas*

18:25 **Ian Quibod** Population genomics of Rice Bacterial Leaf Blight in Africa

18:40 **Johan Quilbé** Mechanisms governing the interaction between root and bacteria in the model legume *L. japonicus*

18:55 **Antinéa Sallen** Genotypic and genomic analyses of French collection of *Ralstonia solanacearum* strains to improve knowledge of outbreak origins

19:10 **Alvaro Perez-Quintero** The evolution of evolvability in Xanthomonas

19:25 **MY POSTER IN 60 SECONDS** Even numbers / numéros **p**airs posters n°50 to 58

20:00 21:00 DINER

21:00 SESSION EVEN NUMBER/ CHIFFRE PAIR POSTERS

Thursday, January 16th

08:45 10:25 SESSION Chemical ecology, secondary metabolites

Moderators: Laure Weisskopf, Florence Wisniewski-Dyé

08:45 **Invited speaker Laure Weisskopf** Chemical communication and its impact on the expression of biocontrol traits in plant-associated bacteria

09:25 **Timothée Zannis-Peyrot** Phytobacteria extracellular vesicles as understimated actors in plant-bacteria interactions

09:40 **Ségolène Bouche** Ecology and mineral weathering ability of the ectomycorrhizosphere strain *Pseudomonas* sp. PML3(3)

09:55 **Eva Caly Simbou** Bacteriocins of the *Ralstonia solacearum* species complex: Biochemical and genetic characterization

10:10 **Coline Amaro-Lauer** Competitive colonization of the potato host by the phytopathogen *Dickeya solani*

10:25 BREAK

10:50 12:30 SESSION Effect of the environment, Epidemiology, Ecology

Moderators: Edward Topp, Marie-Anne Barny

10:50 **Invited speaker Edward Topp** The development and transmission of antibiotic resistance in crop production systems

11:30 **Léna Pesenti** Exploring the xylem-sap to unravel interactions between bacterial xylem endophytes and two phytopathogenic bacteria, *Xylella fastidiosa* and *Brenneria salicis* in *Salicaceae* through metagenomics and *in vitro* studies

11:45 **Gwennaëlle Henry** Impact of an introduced species of alder (*Alnus cordata*) on the associated *Frankia* diversity and nitrogen cycling microbial communities

12:00 **Sylvain Vicente** Deciphering population dynamics of *Ralstonia solanacearum* inside a host tomato

12:15 **Mathilde Hutin** Bacterial Leaf Blight of rice: an emerging threat to rice cultivation in East Africa

12:30 13:30 LUNCH

13:30 17:00 FREE or ROUND TABLE DISCUSSION (according to weather condition)

17:00 19:40 SESSION Plant immunity & Plant Health management: diagnostic, biocontrol

Moderators: Guillaume Chesneau, Barbara Pivato

17:00 **Invited speaker Guillaume Chesneau** The root microbiota: unraveling microbial multi-kingdom metabolic interactions and their implications for plant health

17:40 **Terriane Vanhove** Diversity of molecular factors of resistance to bacterial wilt (*Ralstonia solanacearum*) in eggplant

17:55 **Annaëlle Baud** Phage patrol: an effective targeted biocontrol strategy against *Xanthomonas hortorum* pv. *vitians*

18:10 **Soline Marty** Water and immunity – using a novel plant screen to identify candidate genes

18:25 **Valérie Martin** Fungal leaf communities reduce the development of grapevine downy mildew

18:40 **Michel Barbier** Description of the bacterial and fungal microbiota of rice field soils with antagonistic effects against the rice root-knot nematode *Meloidogyne graminicola* in Cambodia

18:55 **Audrey Pécourt** Towards a more sustainable agriculture, *Sphingomonas sediminicola* a potential bio-inputs to reduce chemicals inputs in a local crop rotation

19:10 **Claire Prigent-Combaret** Developing biocontrol products against broomrapes: from lab to field

19:45 20:45 DINER

21:00 PARTY TIME !

Friday, January 17th

09:00 09:30 SESSION Chemical ecology, secondary metabolites

Moderators: Florence Hommais, Benoit Alunni

09:00 **Aurélie Deveau** Roles of salicylates in the regulation of poplar microbiome colonization: from defence signalling molecule to carbon source

09:15 **Frédérique Reverchon** Antifungal and plant growth promoting activity of avocado phyllosphere bacteria and their organic extracts

09:30 10:00 SESSION Structure, function and engineering of the microbiome

Moderators: Wafa Achouak, Frédéric Labbé

09:30 **Yvan Moënne-Loccoz** Geographic comparison of soils suppressive to root disease

09:45 **Clara Torres-Barceló** The phageome of apricot trees and its association with bacterial canker disease

10:30 Prize distribution and closing ceremony

12:00 13:00 LUNCH

Introductive session

Invited speaker: Olaya Rendules

Moderators: Marie Simonin, Benoît Alunni

The bacterial capsule as a key driver of microbial evolution

Olaya RENDUELES (1)

(1) Laboratoire de Microbiologie et Génétique Moléculaires (LMGM), CNRS UMR5100, Centre de Biologie Intégrative (CBI), Université de Toulouse, CNRS, Université Toulouse III - Paul Sabatier (UT3), Toulouse, France

Surface structures are the first cellular components to interact with the environment and thus should have a major impact in bacterial fitness and play a prominent role in adaptation and in bacteria-host interactions. One such structure, the bacterial capsule, encoded in more than half of the bacterial species (1), including many facultative pathogens but more surprinsingly many soil organism. Capsules are best known for their role in clinical settings, and are considered a major virulence factor. However, capsules also play an important role outside a host because they protect the cells from physical and chemical stresses.

In our laboratory, we use Klebsiella pneumoniae as a model species, a gut commensal but also an ubiquitous bacterium, also found in the soil. We combine molecular genetics, experimental evolution and comparative genomics to decipher how the capsule evolves and in turn drives the adaption to novel environments, including the soil.

References:

(1) Rendueles, O., Garcia-Garcerà, M., Néron, B., Touchon, M. & Rocha, E. P. C. Abundance and co-occurrence of extracellular capsules increase environmental breadth: Implications for the emergence of pathogens. PLoS Pathog. 13, e1006525 (2017).

SESSION Molecular dialog during plant-bacteria interactions: from symbiosis to pathogenicity

Invited speaker: Adam Schikora

Moderators: Adam Schikora, Axel de Zélicourt

Specific and general factors modulating plant response to bacterial N-acyl homoserine lactones

Yongming DUAN (1), Matthias CAMBEIS (1) Min HAN (1), Maja GRIMM (1) and Adam SCHIKORA (1)

(1) Julius Kühn Institute (JKI) - Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Messeweg 11/12, 38104 Brunswick, Germany

Plants can perceive bacterial molecules such as the quorum sensing signals N-acyl homoserine lactones (AHL), thus modifying their fitness in response to environmental factors. Even though the benefits conferred by AHL depend on various hormone signaling pathways, the understanding of AHL signaling, especially the response to AHL presence, remains largely unknown. To fil this gap we used weighted gene co-expression network analysis, multi-omics network analysis, and reverse transcription quantitative PCR (RT-qPCR) assays to identify key genes in AHL signaling. To obtain such comprehensive insights into plant AHL-signaling, we integrated available transcriptome data from Arabidopsis thaliana exposed to different single or multiple AHL molecules and performed a weighted gene co-expression network analysis. We identified several key genes regulated in plants exposed to multiple AHL molecules. Multi-omics network analysis and RT-qPCR assay revealed a potential role of WRKY transcription factors. Results presented here offer good indications for exploring the mechanism of plants' response to bacterial signaling molecules, which could further support the application of AHL-producing bacteria in sustainable agriculture, for example the cultivation of barley, a model crop plant used in our studies.

References:

Duan et al. (2024) Network analysis uncovers the master role of WRKY transcription factors in Arabidopsis thaliana response to N-acyl homoserine lactones. CABI Agriculture and Bioscience (2024) 5:6 https://doi.org/10.1186/s43170-023-00206-x

New insights into the T3SS-triggered nodulation between Bradyrhizobium and Aeschynomene

CAMUEL Alicia, GIRAUD Eric

Some Bradyrhizobia have the capacity to nodulate Aeschynomene species in the absence of Nod factor, thanks to their Type 3 Secretion System. It has been identified in a diversity of Bradyrhizobium strains that Type 3 Effectors, such as ErnA and Sup3, trigger nodule organogenesis. However, the plant signalling pathways they activate remain unknown. To better understand this process, we first investigated the mode of action of ErnA by performing transcriptomic analysis of transgenic roots either overexpressing or not overexpressing ernA. In parallel, we analysed the symbiotic properties of a selection of Bradyrhizobium strains with different sets of effectors on A. evenia mutants altered in various symbiotic signalling genes.

I will present here potential genes under the control of ErnA, including the transcription factor AeNF-YC, which emerged from the comparative transcriptomic analysis. Furthermore, I will show that depending on the set of effectors secreted by the bacteria, certain canonical symbiotic determinants (POLLUX, CCaMK and CYCLOPS) can be dispensable for the nodulation. In contrast, NIN and NSP2 are required for T3SS-triggered nodulation. Taken together, these results highlight the diversity of the nodulation process and the mechanisms by which T3SS-dependent nodulation is achieved in legumes.

Xanthomonas campestris pv. campestris Tal12a contribution to black rot disease and prediction of its host targets in cauliflower

Brice CHARLEUX(1), Carine GRIS (1), Sébastien CARRERE (1), Alvaro L. PEREZ-QUINTERO (2), Aurélie Le Ru (3), Caroline BELLENOT (1), Ivanna FUENTES (1), Zoë E. DUBROW (4), Adam J. BOGDANOVE (4), Laurent D. NOEL (1) and Corinne AUDRAN (1)

(1) LIPME, Université de Toulouse, INRAE, CNRS, Université Paul Sabatier, Castanet-Tolosan, France

(2) PHIM, Université de Montpellier, IRD, CIRAD, INRAE, Institut Agro, Montpellier, France

(3) Institut Fédératif de Recherche 3450, Plateforme Imagerie, Pôle de Biotechnologie Végétale, CNRS, Université Paul Sabatier, Castanet-Tolosan, France

(4) Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, U.S.A

Xanthomonas campestris pv. campestris (Xcc) bacterial pathogen is the causal agent of black rot disease on Brassica crops. One of the virulence factors of Xcc is the type three Transcription Activator-Like Effector (TALE) protein Tal12a which is prevalent in this pathogen. TALE proteins are injected inside plant cells and nuclei where they modulate host gene expression. In this study, we show that Tal12a promotes virulence and proliferation in cauliflower. Transcriptomic analysis identified 380 cauliflower genes that were upregulated in response to Xcc strains expressing Tal12a. Among these, nine genes, including transcription factors, auxin-related genes and clade III SWEET genes, were identified as candidate targets of Tal12a. Tal12a-binding element predictions combined with heterologous expression assays in Nicotiana benthamiana confirmed that Tal12a directly targets BoCYP450, BoIAA7a/b, BoATHB-X, BoTIC, and BoERF043. Though BoSWEET13 and BoSWEET14c were also upregulated in presence of Tal12a, the absence of predicted TALE-binding elements suggests an indirect regulation which contrasts with the direct activation observed in other pathosystems. These findings not only evidence the complexity of Tal12a-mediated gene regulation in cauliflower but also identify the first candidate TALE targets in this plant.

Function and adaptation of symbiotic receptors that activate the Nod-independent symbiosis in the tropical legume Aeschynomene evenia

Laura Agert1, Natasha Horta Araújo2, Marjorie Pervent1, Maëlle Rios2, Frédéric Gressent1 and Jean-François Arrighi2

1 Plant Health Institute of Montpellier (PHIM), INRAE, Campus International de Baillarguet, Montpellier, France

2 Plant Health Institute of Montpellier (PHIM), IRD, Campus International de Baillarguet, Montpellier, France

Leguminous plants form nitrogen-fixing symbiosis with rhizobia typically via Nod factor (NF) recognition by LysM-RLK receptors, activating their co-receptor SYMRK for signaling. However, in Aeschynomene evenia, some photosynthetic Bradyrhizobia nodulate without using NF, referred to as the Nod-independent symbiosis. A forward genetic screen using ethyl methane sulfonate (EMS) mutagenesis in A. evenia (1) identified nodulation mutants, several of them being mutated in receptor-encoding genes, but not in LysM-RLK genes, which show adaptations in the A. evenia genome. This study revealed partial conservation of the common signalling pathway seen in model legumes, with the notable finding that SYMRK exists in two copies in A. evenia genome.

Understanding the recent evolutionary history of these receptors is important to unveil the molecular mechanisms intricate to this Nod-independent symbiosis. To achieve this, a comparative analysis is underway, using A. patula, a Nod-dependent species closely related to A. evenia (2). It is hypothesized that NF receptor orthologs in A. evenia, AeNFP and AeLYK3, are not involved in Nod-independent symbiosis. Though recent preliminary results suggest a more complex outcome. NF co-receptor, AeSYMRK, seems to be involved in the Nod-independent process (3). Yet, its characterization is complexified by the actual presence of two genomic copies that are likely to be functionally redundant in A. evenia. Promoter-GUS studies showed that these two copies are expressed throughout nodulation and their overexpression induced spontaneous nodulation, as previously observed in model legumes (4). Together, these results suggest that both AeSYMRK copies play an important role in A. evenia symbiosis.

Ongoing CRISPR-Cas9 knock-outs of these genes will clarify their role in A. evenia symbiosis. Ultimately, providing key insights into the molecular mechanisms of Nod-independent symbiosis.

References

1. Quilbé, J., Lamy, L., Brottier, L. et al. (2021). Genetics of nodulation in Aeschynomene evenia uncovers mechanisms of the rhizobium–legume symbiosis. Nat Commun, 12, 829.

2. Brottier, L., Chaintreuil, C., Simion, P. et al. (2018). A phylogenetic framework of the legume genus Aeschynomene for comparative genetic analysis of the Nod-dependent and Nod-independent symbioses. BMC Plant Biol. 18, 333.

3. Fabre, S., Gully, D., Poitout, A., Patrel, D., Arrighi, J. F., Giraud, E., ... & Cartieaux, F. (2015). Nod factor-independent nodulation in Aeschynomene evenia required the common plant-microbe symbiotic toolkit. Plant Physiology, 169(4), 2654-2664.

4. Ried, MK., Antolín-Llovera, M., Parniske, M. (2024). Spontaneous symbiotic reprogramming of plant roots triggered by receptor-like kinases. eLife 3:e03891.

Characterization of the role of apple agglutinins in resistance against fire blight

Axelle FRANTZ (1), Matthieu GAUCHER (1), Marie-Noëlle BRISSET (1), Alexandre DEGRAVE (1)

(1) Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, 49000, Angers, France

Erwinia amylovora (Ea) is responsible for fire blight, a bacterial disease that affects apple and pear orchards by causing necrosis that leads to the death of the tree. Despite the damage caused, apple defense mechanisms against this pathogen are poorly described. We identified a new family of genes involved in defense against Ea that encodes lectins named Malus domestica agglutinins (MdAGGs or apple agglutinins). We aim to understand the mode of action of these proteins with different in vivo and in vitro approaches and we demonstrate that the recombinant MdAGG is able to agglutinate Ea cells in vitro. In planta, bacteria quickly die off after being agglutinated but we show that MdAGGs do not have any bactericidal properties. Our studies are now driven by the hypothesis that these proteins interact with (an)other protein(s), which could have bactericidal properties. The latest data dedicated to the understanding of plant defenses toward Ea involving MdAGGs will be presented.

Mechanisms by which plant and synthetic small non-coding RNAs direct gene silencing in bacteria

Liam LEBEAU(1), Lionel NAVARRO(1)

(1) Institut de Biologie de l'Ecole Normale Supérieure (IBENS), Centre National de la Recherche Scientifique (CNRS), Institut National de la Santé et de la Recherche Médicale (INSERM), Université de recherche Paris, Sciences & Lettres (PSL), Paris, France.

Small non-coding RNAs (sRNAs) involved in RNA interference can be transported between two interacting organisms, thereby triggering trans-species gene silencing. This cross-kingdom RNAi process has been extensively studied in plant-fungal interactions, and occurs in a bidirectional manner, as part of antifungal immune and fungal virulence responses (1-6). Intriguingly, we have recently demonstrated that Arabidopsis transgenic plants expressing sRNAs directed against key virulence factors of Pseudomonas syringae pv. tomato strain DC3000 (Pto DC3000), can trigger gene silencing and the dampening of pathogenesis (7). Three populations of active extracellular sRNAs were recovered in the apoplast of these transgenic plants. The first one is non-vesicular and associated with proteins, whereas the second one is located inside extracellular vesicles. The third population is unbound to proteins and in a dsRNA form. However, the mode of action of these sRNAs over their cognate targets, and the machinery responsible for gene silencing in Pto DC3000, remain unknown. To address this, we have established an in vitro system that recapitulates this gene silencing phenomenon in Pto DC3000 using chemically synthesized sRNAs. Using this approach, we found that 21 nt long sRNAs, in a dsRNA form, and composed of 2-nt 3' overhangs, to mimic Dicer-Like (DCL)-dependent products, or that are blunt ended, were effective in AGS. This was also true with sequence complementary single-stranded sRNAs (ssRNAs) that were found active against stress-responsive virulence factors. The latter ssRNAs were also shown to be effective against essential genes, which are constitutively expressed and critical for bacterial fitness. I will present the results from these analyses. I will also report on the different approaches currently developed to identify and characterize the machinery responsible for sRNA-directed gene silencing in bacteria.

References:

1. Cai, Q. et al. Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. Science 360, 1126–1129 (2018).

2. Zhang, T. et al. Cotton plants export microRNAs to inhibit virulence gene expression in a fungal pathogen. Nature Plants 2, (2016).

3. Wang, M. et al. Bidirectional cross-kingdom RNAi and fungal uptake of external RNAs confer plant protection. Nat Plants 2, 16151 (2016).

4. He, B. et al. RNA-binding proteins contribute to small RNA loading in plant extracellular vesicles. Nat. Plants 7, 342–352 (2021).

5. Koch, A. et al. Host-induced gene silencing of cytochrome P450 lanosterol C14α-demethylase–encoding genes confers strong resistance to Fusarium species. PNAS 110, 19324–19329 (2013).

6. Koch, A. et al. An RNAi-Based Control of Fusarium graminearum Infections Through Spraying of Long dsRNAs Involves a Plant Passage and Is Controlled by the Fungal

Silencing Machinery. PLoS Pathog 12, (2016).

7. Ravet, Zervudacki et al. Vesicular and non-vesicular extracellular small RNAs direct gene silencing in a plant-itneracting bacterium. doi: https://doi.org/10.1101/863902

Characterization of an adaptive mutation in a Type 6 effector that improves the proliferation of Ralstonia solanacearum in Mimosa pudica nodules

Marvin NAVARRO (1), Saida MOUFFOK (1), Minxing Tang (1), Thibault SANA (2), Alice GUIDOT (1), Delphine CAPELA (1)

1. LIPME, Université de Toulouse, INRAE, CNRS, Castanet-Tolosan 31320, France

2. Institut Européen de Chimie et Biologie (IECB), Pessac 33607, France

Evolution has enabled certain bacteria to establish a spectrum of associations with eukaryotes, ranging from parasitism to mutualism. These changes in lifestyle are the result of complex multifactorial mechanisms, which may require specific functions imposed by the host or by the new lifestyle. At the molecular level, these changes often result from the acquisition of genes by horizontal transfer, as well as the modulation of the expression of endogenous genes. In order to study the molecular mechanisms underlying the emergence of new symbiotic strains observed in nature, an evolutionary experiment was carried out on the plant pathogenic bacterium Ralstonia pseudosolanacearum. The aim was to convert its reference strain, GMI1000, into a mutualistic symbiont of the legume Mimosa pudica by transferring essential symbiotic genes from the natural symbiont Cupriavidus taiwanensis and evolving the resulting strain in contact with the plant. The first stages of symbiosis, nodulation and intracellular infection, were rapidly acquired and then strongly improved after a few cycles of nodulation. We then wondered which endogenous functions were modulated to carry out these initial stages of symbiosis. Throughout this adaptive process, numerous mutations occurred in various genes, with a high frequency in those encoding structural components and putative effectors of the type VI secretion system (T6SS). Among these mutations, one missense mutation that appeared in the RSp0178 gene, encoding a type VI effector, is responsible for a significant increase in bacterial proliferation in nodules. This mutation is equivalent to a loss of function but does not alter the secretion of this effector in vitro. Moreover, preliminary data suggest that RSp0178 is also secreted during the symbiotic interaction although this result needs to be confirmed. Its secretion in nodules might induce defense reactions in the plant, thereby blocking bacterial proliferation.

References:

1. Drew, G. C., Stevens, E. J., & King, K. C. (2021). Microbial evolution and transitions along the parasite–mutualist continuum. Nature Reviews Microbiology, 19(10), 623-638.

2. Doin de Moura, G. G., Remigi, P., Masson-Boivin, C., & Capela, D. (2020). Experimental evolution of legume symbionts: what have we learnt?. Genes, 11(3), 339.

3. Marchetti, M., Capela, D., Glew, M., Cruveiller, S., Chane-Woon-Ming, B., Gris, C., ... & Masson-Boivin, C. (2010). Experimental evolution of a plant pathogen into a legume symbiont. PLoS biology, 8(1), e1000280.

4. Capela, D., Marchetti, M., Clérissi, C., Perrier, A., Guetta, D., Gris, C., ... & Masson-Boivin, C. (2017). Recruitment of a lineage-specific virulence regulatory pathway promotes intracellular infection by a plant pathogen experimentally evolved into a legume symbiont. Molecular biology and evolution, 34(10), 2503-2521.

The role of anaerobic respiration of carbon sources in the adaptation and survival of Dickeya dadantii

Clara BLONDE (1)*, Amandine M'SAKNI (1), Sarah JOLY (1), Isabelle RAHIOUI (2), Baudoin DELEPINE (3), Rémi PEYRAUD (3), William NASSER (1), Sylvie REVERCHON (1), Céline BROCHIER (4), Feth el Zahar HAICHAR (1)

(1) Microbiologie, Adaptation et Pathogénie – UMR 5240, Villeurbanne, France

(2) Biologie Fonctionnelle Insectes et Interaction – UMR 0203, Villeurbanne, France

(3) iMean, Toulouse, France

(4) Laboratoire de Biométrie et Biologie Évolutive - UMR CNRS 555, Villeurbanne, France

Plant pathogenic bacteria face many specific challenges when colonising and/or infecting plants to acquire nutrients. In the case of the bacterium Dickeya dadantii, which is capable of colonising different plants and living in the anoxic environments of the rhizosphere and plant tissues, the ability to grow in anoxic conditions is an adaptive trait that favours its development and dissemination. We hypothesise that the ability to respire anaerobically the carbon (C) compounds present in its environment is a physiological advantage for phytopathogenic bacteria. Our objective is to demonstrate the ability of Dickeya dadantii to anaerobically respire carbon compounds present in the apoplast. Among the terminal electron acceptors that we have identified, asparagine enabled the greatest growth in vitro. A metabolic model was used to predict in silico the asparagine respiration pathway. Mutants of the asparagine respiration pathway genes were constructed and their impact on virulence in planta was evaluated in order to highlight the role of anaerobic respiration in Dickeya dadantii infection.

References:

Paul G. Dennis, Anthony J. Miller, Penny R. Hirsc (2010). Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities?

Arantza Rico and Gail M. Preston (2008). Pseudomonas syringae pv. tomato DC3000 Uses Constitutive and Apoplast-Induced Nutrient Assimilation Pathways to Catabolize Nutrients That Are Abundant in the Tomato Apoplast

Role of the CckA-ChpT-DivL complex in the phosphorylation of the master regulator CtrA during the cell cycle and nitrogen-fixing symbiosis in Sinorhizobium meliloti

Roza Mohammedi (1), Sara Dendene (1), Emanuele G. Biondi (1)

(1) Institute for Integrative Biology of the Cell, 91190 Gif-sur-Yvette, France

Sinorhizobium meliloti is an alphaproteobacterium, which is able to live free in the soil or in symbiosis with legumes. During symbiosis, bacteria fix atmospheric nitrogen within symbiotic organs, called nodules, where they undergo cell differentiation into bacteroid. The transcription factor CtrA has been shown to be the master regulator of the cell cycle and bacteroid development is accompanied by a gradual disappearance of CtrA, suggesting that CtrA plays a crucial point for the establishment of the symbiosis. In the alphaproteobacterium Caulobacter crescentus, related to S. meliloti, the cell cycle is also regulated by CtrA. CtrA is regulated by a phosphorelay system consisting of the two histidine kinases DivL and CckA and the histidine phosphotransferase ChpT. Orthologs of these different regulators are present in S. meliloti, suggesting a conservation of this module in the regulation of CtrA in this bacterium.

In this work the functions of the CckA-ChpT-DivL complex and its impact on CtrA in S. meliloti in free and symbiotic life have been studied. We demonstrated that divL is an essential gene involved in the proper functionning of the cell cycle and in the regulation of CtrA. We also purified the phosphorelay proteins and reconstructed a part of the phosphorylation cascade in-vitro. Finally, the DivL-depletion strain was not able to perform an efficient symbiotic relationship with Medicago sativa under the tested conditions. In conclusion, the phosphorelay system of CtrA phosphorylation plays an esential role in cell cycle regualtion and development of bacteroids.

References:

Alunni, B., Gourion, B., 2016. Terminal bacteroid differentiation in the legume−rhizobium symbiosis: nodule-specific cysteine-rich peptides and beyond. New Phytol. 211, 411–417. https://doi.org/10.1111/nph.14025

Biondi, E.G., Reisinger, S.J., Skerker, J.M., Arif, M., Perchuk, B.S., Ryan, K.R., Laub, M.T., 2006. Regulation of the bacterial cell cycle by an integrated genetic circuit. Nature 444, 899–904. https://doi.org/10.1038/nature05321

Pini, F., De Nisco, N.J., Ferri, L., Penterman, J., Fioravanti, A., Brilli, M., Mengoni, A., Bazzicalupo, M., Viollier, P.H., Walker, G.C., Biondi, E.G., 2015. Cell Cycle Control by the Master Regulator CtrA in Sinorhizobium meliloti. PLoS Genet. 11. https://doi.org/10.1371/journal.pgen.1005232

Xue S & Biondi EG (2019) Coordination of symbiosis and cell cycle functions in

Sinorhizobium meliloti. Biochim. Biophys. Acta BBA - Gene Regul. Mech. 1862, 691–696.

MilliDrop: Unlocking Insights into Plant-Bacteria Interactions

Marie GROSSELIN - Alex LHEUREUX

Gold Standard Diagnostics Millidrop, Massy, France

Understanding plant-bacteria interactions is essential for advancing sustainable agriculture, improving crop resilience, and enhancing soil health. MilliDrop's innovative droplet-based millifluidic technology provides a powerful platform for studying these complex relationships with high precision and efficiency.

MilliDrop encapsulates bacterial cells in microliter-sized droplets, creating thousands of individual microenvironments. These droplets act as miniaturized bioreactors, enabling the cultivation, tracking, and real-time analysis of bacterial growth and behavior under diverse conditions. By introducing plant-derived compounds or mimicking rhizosphere environments, researchers can use MilliDrop to investigate how bacterial strains interact with plants, respond to stress, or produce beneficial metabolites.

The system's high-throughput capabilities allow for simultaneous testing of hundreds of conditions, generating robust datasets with minimal sample volumes. Its precise environmental control is ideal for screening microbial strains for plant growth promotion or biocontrol potential. The droplet-based approach not only enhances reproducibility but also accelerates the discovery of novel insights into plant-microbe dynamics.

MilliDrop's technology equips researchers with a scalable, efficient tool for exploring the microbial contributions to plant health and productivity. By bridging innovation with agricultural research, MilliDrop supports the development of sustainable solutions for global food security challenges.

SESSION Structure, function and engineering of the microbiome

Invited speaker: Samuel Jacquiod

Moderators (Tuesday 14th): Samuel Jacquiod, Corinne Vacher

Moderators (Friday 17th): Wafa Achouak, Frédéric Labbé

Artificial selection of rhizosphere microbiota altering plant phenotype: treating microbial communities as units of selection

Samuel JACQUIOD (1), Manuel BLOUIN (1)

(1) Agroécologie, Institut Agro Dijon, INRAE, Université Bourgogne, Dijon, Franche-Comte, France

Artificial selection of entire rhizosphere microbiota is an experimental evolution approach relying on the iterative selection and inoculation of microbial communities associated to plants displaying a phenotype of interest. Using this approach, we have selected rhizosphere microbiota of Brachypodium distachyon associated with high or low leaf greenness, a proxy of plant performance, in a sandy soil. We showed that the selection process is undergoing two phases: an initial transitory phase with no detectable effects, and a stabilization phase where the microbiota structure stabilized, concomitantly with heritability in leaf greenness. We showed a remarkable correlation between the variability in plant traits and selected microbiota structures, revealing distinct microbial sub-communities associated with high or low leaf greenness. Then, we aimed to test the reproducibility of our microbiota-induced effects on the greenness of different plant species grown in different soils. We thus inoculated the evolved microbiota on different Poaceae species (Avena strigosa, Triticum aestivum, Zea mays; Brachypodium distachyon was used as a positive control), either in the same sandy soil used during artificial selection, or in a contrasting clayey soil. We found that the effects of our selected microbiota are species-dependent, as we could not reproduce the effects on the three Poaceae species. However, we could reproduce the effects on Brachypodium distachyon in the two different soils. We identified the presence of a soil independent microbial sub-community that is always associated with higher greenness in Brachypodium distachyon. Our result show that artificial selection of rhizosphere microbiota can be used to rapidly obtain evolved communities able to alter the plant phenotype in the intended way, and under varying pedological conditions. This may have consequences for futur plant breeding programs, as the inclusion of microbiota in such programs may prove usefull.

References:

Jacquiod, S., Spor, A., Wei, S., Munkager, V., Bru, D., Sørensen, S. J., Salon, C., Philippot, L., Blouin, M. (2022). Artificial selection of stable rhizosphere microbiota leads to heritable plant phenotype changes. Ecol. Lett. 25, 189–201. doi: 10.1111/ele.13916

Jacquiod, S., Nesme, J., Ducourtieux, C., Pimet, E., Blouin, M. (2024). Artificially selected rhizosphere microbiota modify plant growth in a soil-independent and species-dependent way. Plant Soil (in press). https://doi.org/10.1007/s11104-024-06947-6

Multi-Kingdom Synthetic Communities Modulate Seedling Microbiota and Reveal Key Bacteria-Fungi Interactions in Brassica napus

SUTEAU Logan (1), MARAIS Coralie (1), HELLYN Kaat (1), SIMONIN Marie (1), CAMPION Claire (1), GUSCHINSKAYA Natalia (1)

(1) Institut de Recherche en Horticulture et Semences (IRHS), UMR 1345, Université d'Angers / INRAE / Institut Agro ; 49070 Beaucouzé, France.

Synthetic Communities (SynComs) are new tools used to manipulate plant microbiota to improve plant health or study microbial colonization processes. While the majority of research in this field has focused on bacterial SynComs (1), multi-kingdom communities provide new insight into community interactions and show great biocontrol potential (2). This study presents the impact of Brassica napus seed inoculation with 20 different multi-kingdom SynComs composed of bacteria, filamentous fungi and yeast and constructed with both a priori selection (based on abundance, growth rate...) and random selection. This selection resulted in a total of 24 bacterial strains (20 different genera), 10 filamentous fungi (8 different genera) and 11 yeasts (6 different genera), each present in 2 to 11 different SynComs. The initial stage of the process involved optimising the inoculation of seeds using alginate coating, which resulted in a two-log increase in the concentration of inoculated microorganisms. The inoculated SynComs demonstrated the capacity to modulate seedling microbiota, explaining 33% of the variance for bacterial community structure and 22% for fungal communities. Bacterial and fungal strains with high transmission rates to seedlings were identified (Pantoea agglomerans, Pseudomonas putida, Alternaria sp., Vishniacozyma sp.). However, the transmission of the majority of strains was found to be significantly influenced by the composition of the SynCom. The inoculation of SynComs had an impact on seedling emergence and growth rates. Interestingly, the phenotypic effects of some strains depended on the identity of the other SynCom members. For example, Pseudomonas baetica was transmitted in seedling in 4 SynComs but exerted a negative effect in only 3 of them. This suggests the existence of biotic interactions between the strains during the process of seedling colonization. This study demonstrates that seed inoculation with a multi-kingdom SynCom results in the engineering

References:

1. Arnault, G. et al. Seedling microbiota engineering using bacterial synthetic community inoculation on seeds. FEMS Microbiology Ecology 100, fiae027 (2024).

2. Zhou, X. et al. Cross-kingdom synthetic microbiota supports tomato suppression of Fusarium wilt disease. Nat Commun 13, 7890 (2022).

Population dynamics modelling of synthetic communities isolated from seed bean microbiota and their impact on pathogen Xanthomonas citri pv. fuscans

Amélie CADDEO (1, 2), Matthieu BARRET (1), Rémi PEYRAUD (2)

(1) INRA, UMR1345 Institut de Recherche en Horticulture et Semences, Beaucouzé, France

(2) iMEAN, Toulouse, France

The seed microbiota plays a key role in germination and seedling emergence. Modifying its composition by inoculating seeds with synthetic communities (SynComs) is one strategy to improve crop establishment. However, microbiota's interactions complexity is a challenge encountered, which can be overcome by mathematical modelling and systems biology. This study proposes a modeling approach to understand how seed bean microbiota reduces the invasion of the plant pathogen Xanthomonas citri pv. fuscans (Xcf) based on trophic competition. Genome-scale metabolic models of Xcf and ten bacterial strains representative of the seed bean microbiota have been reconstructed with a semi-automatic pipeline (Peyraud et al. 2016) followed by a deep manual curation. Metabolic pathways were validated by comparing Biolog PM plates results with model simulations, showing model accuracy ranging from 70.3% to 91.8%. Moreover, a population dynamics algorithm based on dFBA method (Mahadevan et al. 2002) using the individual models has been constructed. To validate the population dynamics simulations, growth of individual strains on minimal media supplemented with different carbon sources were monitored. The average difference between the simulations and experimental data varies from 6.1 to 68.6%. Finally, trophic competition between Xcf and the selected strains was studied by simulating population dynamics at the community level. Preliminary simulations showed that some SynComs can reduce the XCF biomass until 67%, while others increase it until 20%. To validate these predictions, the impact of 14 SynComs, each combining 5 strains including GFP-tagged Xcf, on Xcf growth was monitored. Then, predicted interactions were analyzed between SynComs and Xcf based on trophic competition mechanism. The models will be used in high-throughput simulations to predict which SynComs best decrease the implantation of Xcf and then will be validated first in vitro into seed bean exudate and finally in planta.

References:

Mahadevan, Radhakrishnan, Jeremy S. Edwards, et Francis J. Doyle. 2002. « Dynamic Flux Balance Analysis of Diauxic Growth in Escherichia Coli ». Biophysical Journal 83 (3): 1331-40. https://doi.org/10.1016/S0006-3495(02)73903-9.

Peyraud, Rémi, Ludovic Cottret, Lucas Marmiesse, Jérôme Gouzy, et Stéphane Genin. 2016. « A Resource Allocation Trade-Off between Virulence and Proliferation Drives Metabolic Versatility in the Plant Pathogen Ralstonia Solanacearum ». Édité par Darrell Desveaux. PLOS Pathogens 12 (10): e1005939. https://doi.org/10.1371/journal.ppat.1005939.

Revealing microbial consortia that interfere with grapevine downy mildew through microbiome epidemiology

Paola FOURNIER, Lucile PELLAN, Aarti JASWA, Marine CAMBON, Alexandre CHATAIGNER, Olivier BONNARD, Marc RAYNAL, Christian DEBORD, Charlotte POEYDEBAT, Simon LABARTHE, François DELMOTTE, Patrice THIS, Corinne VACHER

INRAE, Centre de Recherches de Bordeaux-Nouvelle Aquitaine

Background

Plant and soil microbiomes can interfere with pathogen life cycles, but their influence on disease epidemiology remains understudied. Here, we analyzed the relationships between plant and soil microbiomes and long-term epidemiological records of grapevine downy mildew, a major disease caused by the oomycete Plasmopara viticola.

Results

We found that certain microbial taxa were consistently more abundant in plots with lower susceptibility to the disease and that microbial community composition could predict disease susceptibility. Microbial diversity was not strongly linked to epidemiological records, suggesting that susceptibility is more related to the abundance of specific microbial taxa. These key taxa were identified in the topsoil, where the pathogen's oospores overwinter, and in the phyllosphere, where zoospores infect leaves. By contrast, the leaf endosphere, where the pathogen's mycelium develops, contained few taxa of interest. Surprisingly, the soil microbiota was a better predictor of downy mildew symptoms than the leaf microbiota, suggesting a significant role of the soil microbiome in this primarily aerial disease.

Conclusion

Our study integrates long-term epidemiological data with microbiome profiles of healthy plants to reveal fungi and bacteria relevant for the biocontrol of grapevine downy mildew. The resulting database provides a valuable resource for designing microbial consortia with potential biocontrol activity. The framework can be applied to other crop systems to guide the development of biocontrol strategies and reduce pesticide use in agriculture.

Cry for help: A differential rice root-associated microbiome response to foliar infection by two pathogenic fungi

Léa JOBERT (1), Lionel MOULIN (1), Gilles BENA (1)

(1) PHIM Plant Health Institute, IRD, CIRAD, INRAE, Institut Agro, Univ Montpellier, Montpellier, France

Increasing evidence suggests that plants may recruit beneficial microbes in their rhizosphere to suppress soil-borne pathogens, but the processes underlying microbiome assembly in response to foliar pathogen infection, as well as the functions in infected hosts, are not fully understood. The cry for help hypothesis suggests that plants can recruit beneficial microbiota to fight against various stresses through the exudation of specific root molecules1. Research on the cry for help hypothesis on rice is limited, but some studies have shown that rice bacterial leaf blight drives rhizosphere microbial assembly and revealed changes in root-associated microbial community following foliar pathogenic infection by Pyricularia oryzae in rice2,3. To our knowledge, no studies have focused on comparing the root-associated microbiota assembly following different foliar pathogenic infections. To provide a comprehensive view of the rice-associated microbiome following a foliar infection, we compared bacterial and fungal communities of root-associated microbiota in healthy rice and those one week after being infected with Bipolaris oryzae (Brown Spot) or two weeks after infection by Pyricularia oryzae (Blast) in greenhouse conditions. Detailed examination of individual taxa revealed that some of these taxa enriched in root-associated microbiome of diseased plants are known to promote plant growth or have antagonistic activities against pathogens. For example, Rhizophagus irregularis, a well described arbuscular mycorrhizal fungi was found enriched in brown spot diseased rice plants compared to healthy ones4. A biocontrol assay will be conducted to assess whether R. irregularis can confer brown spot resistance in the host plant. Interestingly, the enriched taxa were mostly different between the two pathosystems. These initial findings, derived from a single cycle of microbiome adaptation to pathogenic stress, are promising and suggest that there is much to be explored in this area.

References:

1. Rolfe, S. A., Griffiths, J. & Ton, J. Crying out for help with root exudates: adaptive mechanisms by which stressed plants assemble health-promoting soil microbiomes. Curr. Opin. Microbiol. 49, 73–82 (2019).

2. Jiang, H. et al. Rice bacterial leaf blight drives rhizosphere microbial assembly and function adaptation. Microbiol. Spectr. 11, e01059-23 (2023).

3. Dastogeer, K. M. G., Yasuda, M. & Okazaki, S. Microbiome and pathobiome analyses reveal changes in community structure by foliar pathogen infection in rice. Front. Microbiol. 13, 949152 (2022).

4. Guigard, L., Jobert, L., Busset, N., Moulin, L. & Czernic, P. Symbiotic compatibility between rice cultivars and arbuscular mycorrhizal fungi genotypes affects rice growth and mycorrhiza-induced resistance. Front. Plant Sci. 14, 1278990 (2023).

Transient Seed Microbes, Long-Term Impacts: Seed Microbiota Affect Seedling Phenotype and Microbiome Assembly

Louna Colaert-Sentenac (1), Gontran Arnault (1), Daniel Beucher (1), Anaïs Hardouin (1), Coralie Marais (1), Pascale Sautour (1), Guillaume Tcherkez (1), Béatrice Teulat (1), Élisabeth Planchet (1), Marie Simonin (1)

(1) Institut Agro, INRAE, IRHS, SFR QUASAV, Université d'Angers, F-49000 Angers, France

Seed microbiota is highly diverse and variable across individual seeds[1],[2]. To date, the transmission and impacts of this pioneer microbiota remain poorly understood.

Using synthetic bacterial communities (SynCom) inoculation on Phaseolus vulgaris (common bean) seeds, we investigate the transmission of seed microbiota throughout plant development, from seed to seed, and their influence on key phenotypic traits, in relation to plant metabolome.

Seeds were inoculated with five SynComs, each composed of eight bacteria selected based on their prevalence in seed microbiota and transmission potential as SynComs. Seeds were then sown in non-sterile soil and sampled throughout the plant's life cycle, focusing on early developmental stages. Plant compartments were dissected, and microbial communities were profiled using ITS and gyrB markers. Simultaneously, metabolomic analysis (GC-MS) detected changes in over 345 metabolites during early development, and seedling phenotype was characterized.

During germination and emergence, seed-borne bacteria were primarily transmitted to developing aerial compartments, making up 88%, 80.2%, and 83.2% of the microbiota in cotyledons, leaves, and stems at the seedling stage, respectively. The 30 inoculated strains exhibited differential colonization patterns, with selected Kosakonia, Siccibacter, Curtobacterium and Pantoea agglomerans displaying the highest colonization rates (relative abundance up to 88 %). These seed microbiota reduced the rate of abnormal seedlings among emerged seedlings, a criterion of plant productivity, but did not significantly affect most seedling morphological traits. Metabolome variations associated with developmental stage and SynCom inoculation were observed. Although inoculated seed-borne bacteria were not found after the seedling stage, they shaped adult-plant leaf microbiota structure, explaining 28.1% to 63.1% of the leaf microbiota composition variance.

Overall, seed microbiota transiently colonize above-ground compartments, inducing phenotypic and metabolomic changes with lasting effects on plant microbiota assembly.

Keywords : seed microbiota, transmission, seedling phenotype and metabolome, legacy effects, plant microbiota assembly.

References:

1: Simonin, M., Briand, M., Chesneau, G., Rochefort, A., Marais, C., Sarniguet, A. and Barret, M. (2022), Seed microbiota revealed by a large-scale meta-analysis including 50 plant species. New Phytol, 234: 1448-1463. https://doi.org/10.1111/nph.18037

2: Chesneau, G., Laroche, B., Préveaux, A., Marais, C., Briand, M., Marolleau, B., Simonin, M., Barret, M., Guttman, D. (2022), Single Seed Microbiota: Assembly and Transmission from Parent Plant to Seedling, mBio, Vol. 13, Issue 6, ISSN 2150-7511. https://doi.org/10.1128/mbio.01648-22

Plant-microbe and plant-plant interactions favouring iron content in crop plants

Barbara PIVATO (1), Amélie SEMBLAT (1), Liya ZHU (1), Laure AVOSCAN (1), Angélique BESSON-BARD (1), Laurent OUERDANE (2), Etienne PASCAL-JOURNET (3 ,4), Philippe LEMANCEAU (1), Sylvie MAZURIER (1)

(1) Agroécologie, INRAE, Institut Agro, Université de Bourgogne - Université de Bourgogne Franche-Comté, Dijon, France

(2) Univ Pau & Pays Adour, CNRS, IPREM, E2S UPPA, F-64000 Pau, France

(3) AGIR, Université de Toulouse, INRAE, Castanet-Tolosan, France

(4) LIPME, Université de Toulouse, CNRS, Castanet-Tolosan, France

Legume crops are valuable for agroecological and food transitions. They reduce chemical inputs by fixing atmospheric nitrogen through symbiosis with N-fixing bacteria and provide protein-rich grains that can partially replace animal proteins. However, due to the risk of anemia from this shift, attention is now focused on the iron content in legume grains. Abiotic stresses, such as limited nutrient availability, particularly iron, also affect legume yield and quality. Improving iron nutrition in legume plants is crucial for maintaining crop yields and ensuring adequate iron levels in grains. Plant microbiota and practices like cereal-legume intercropping offer sustainable ways to enhance plant nutrition.

Our study aimed to understand how different pea varieties and pea-wheat intercropping affect the microbiota involved in iron dynamics and to identify microbail groups able to boost iron content in pea plants. Two pea varieties with varying susceptibility to iron chlorosis were grown in three soil types, and root bacterial communities were analyzed for their response to iron stress. The most iron-stress-resistant bacterial groups were then quantified in four pea varieties, grown alone or intercropped with wheat. Synthetic microbial communities were also tested for their impact on pea growth and nutrition.

We identified 13 bacterial families with low susceptibility to iron stress, with their abundance varying by plant variety. Pea-wheat intercropping improved iron nutrition, with one bacterial family playing a key role in iron mobilization. Ongoing research focuses on identifying plant traits and genes that recruit these beneficial microbes, which could be used in breeding to improve legume iron nutrition, yield, and quality.

References:

Semblat, A., Turanoglu, C., Faivre-Primot, C. et al. Impact of pea-wheat intercropping on grain ionome in relation with changes in Pseudomonas spp. and Enterobacterales abundances. Plant Soil (2024). https://doi.org/10.1007/s11104-024-06861-x

Geographic comparison of soils suppressive to root disease

Alix CATRY (1), Danis ABROUK (1), Ana-Isabel SERRANO-MENDOZA (1), Marjolaine REY (1), Christoph KEEL (2), Marie-Lara BOUFFAUD (3), François BUSCOT (3), Adriana GIONGO (4), Kornelia SMALLA (4), Gilles COMTE (1), Daniel MULLER (1), Yvan MOËNNE-LOCCOZ (1)

(1) Univ Lyon, Université Claude Bernard Lyon 1, CNRS, INRAE, VetAgro Sup, UMR5557 Ecologie Microbienne, 43 bd du 11 novembre 1918, F-69622 Villeurbanne, France

(2) University of Lausanne, Department of Fundamental Microbiology, Quartier UNIL-Sorge, CH-1015 Lausanne, Switzerland

(3) Department of Soil Ecology, Helmholtz Centre for Environmental Research - UFZ, Theodor-Lieser-Str. 4, D-06120 Halle/Saale, Germany

(4) Institut für Epidemiologie und Pathogendiagnostik, Julius Kühn-Institut - Bundesforschungsinstitut für Kulturpflanzen, Messeweg 11/12, D-38104, Braunschweig, Germany

In disease-suppressive soils, the microbiota is efficient in controlling soil-borne pathogens infecting plant roots. However, the specific microbiome traits contributing to soil suppressiveness remain poorly understood, especially as these soils occur across diverse regions and cropping systems, hosting distinct microbiota. We hypothesized that disease-suppressive soils from a same pathosystem exhibit common bacterial communities and plant-protective functions. To test this, we cultivated tobacco in greenhouse experiments using soils from Switzerland and Savoie that were either suppressive or conducive to black root rot caused by the fungus Thielaviopsis basicola. Results showed negligible differences in soil mineral composition related to suppressiveness status (suppressive vs conducive), soil type (morainic vs sandstone cambisols), or geography (Switzerland vs Savoie). In contrast, soil metabolomes varied significantly between individual soils, primarily according to soil type and geography. Metabarcoding revealed that fungal and bacterial communities in the tobacco rhizosphere were influenced by individual soils, with suppressiveness status and soil type significantly affecting microbial composition. While several fungal and bacterial taxa were identified as indicators of suppressiveness in Swiss soils and in Savoie soils, only one bacterial taxon (Bosea) was common to both regions. Metagenomic analysis further showed differences in the microbiota between Swiss and Savoie soils, as well as between suppressive and conducive soils, particularly within Swiss soils. Tobacco shoot metabolomes also varied according to individual soils, soil type, and geography, but certain plant metabolites were associated to suppressiveness in both Savoie and Swiss soils. Overall, these results emphasize the influence of geographic and soil-specific factors on microbial communities and soil suppressiveness, highlighting the need for regional approaches when studying disease-suppressiveness.

References:

Todorović, I., D. Abrouk, N. Fierling, M. Kyselková, M.-L. Bouffaud, F. Buscot, A. Giongo, K. Smalla, A. Picot, V. Raičević, J. Jovičić-Petrović, Y. Moënne-Loccoz, D. Muller. 2024. Manure amendments and fungistasis, and their relation to the protection of wheat from Fusarium graminearum. Applied Soil Ecology 201:105506.

Harmsen, N., P. Vesga, G. Glauser, F. Klötzli, C.M. Heiman, A. Altenried, J. Vacheron, D. Muller, Y. Moënne-Loccoz, T. Steinger, C. Keel, D. Garrido-Sanz. 2024. Natural plant disease suppressiveness in soils extends to insect pest control. Microbiome 12:127.

THE PHAGEOME OF APRICOT TREES AND ITS ASSOCIATION WITH BACTERIAL CANKER DISEASE

Chloé FELTIN (1), Quentin LAMY-BESNIER (2) Sylvain PIRY (1), Cindy E. MORRIS (1), Marie-Agnès PETIT (2), Clara TORRES-BARCELÓ (1)(3)

1 INRAE, Pathologie Végétale, F-84140, Montfavet, France

2 Université Paris-Saclay, INRAE, AgroParisTech, MICALIS Unit, 78352 Jouy en Josas, France

3 PHIM Plant Health Institute, Univ Montpellier, INRAE, CIRAD, Institut Agro, IRD, Montpellier, France

clara.torresbarcelo@inrae.fr

While phages have been extensively studied in human and marine contexts, a huge gap exists in plant-associated phage research (1,2). Most plant-related phage research focuses on biocontrol candidates, neglecting ecological and epidemiological questions. Pseudomonas syringae is a highly diverse complex of bacterial species found in a wide range of environments, including plants. Bacterial canker of apricot trees involves several strains of this species complex (3). The diversity and role of phages in this system remains to be elucidated.

The aim of this study is to define the phagome in apricot trees, comparing healthy and diseased tissues. To this end, purification of viral particles and metagenomic analyses were conducted in soil, buds and twigs of apricot trees. Additionally, metabarcoding analyses estimated the diversity of P. syringae strains in the total DNA of these samples.

The viral fraction in our purified samples was 30%, and 28% of complete and high quality phages were assigned to a bacterial host (4). The specific richness of phages in the soil was higher than that of the other tree niches, which were equivalent. The four niches exhibited distinct phage populations, with some phages being ubiquitous across all niches. Inerestingly, we detected the absence of abundant phages of the Actinomycetota and Bacillota phyla in symptomatic twigs, in contrast to the presence of these phages in the other niches. Conversely, there was a significant increase in the relative abundance of Pseudomonas phages in diseased twigs relative to healthy ones.

Buds and asymptomatic twigs had a similar P. syringae population structure, while symptomatic twigs have a distinct signature that sets them apart from other substrates. The soil P. syringae genetic richness is higher than in the other niches. The present study represents a pioneering investigation of the phagome in apricot trees. This could lead to advances in biocontrol and disease surveillance.

References:

1. Leal Rodríguez, C. et al. (2023) The infant gut virome is associated with preschool asthma risk independently of bacteria. Nat Med

2. Bartlau, N. et al. (2022) Highly diverse flavobacterial phages isolated from North Sea spring blooms. The ISME Journal

3. Parisi, L. et al. (2019) Bacteria from four phylogroups of the Pseudomonas syringae complex can cause bacterial canker of apricot. Plant Pathology

4. Roux, S. et al. (2023) iPHoP: An integrated machine learning framework to maximize host prediction for metagenome-derived viruses of archaea and bacteria. PLOS Biology.

SESSION Physiology of plant-bacteria interactions: regulation, epigenetics, metabolites

Invited speaker: Yael Helman

Moderators: Yael Helman, Mathilde Hutin

For better or worse - the effect of interspecies microbial interactions

on disease severity in plants.

Adi GLASS-LIVNE (1), Dharanishanthi VEERAMUTHU (1), Neta ROTEM (1), Ilya KUBLANOV (1), Saul BURDMAN (1) and Yael HELMAN (1)

(1) Department of Plant Pathology and Microbiology, Institute of Environmental Sciences, The Robert H Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel

Bacteria have evolved a large array of signaling pathways that allow them to reprogram their motility in response to their neighboring colonies and the environmental conditions. In this regard we found that when Paenibacillus spp. bacteria are grown on glucose their motility is inhibited, thereby restricting them to a favorable environment. However, when these colonies are grown in proximity to other bacterial species, such as the plant pathogens Xanthomonas perforans or Acidovorax citrulli, this inhibition is overridden and the Paenibacillus cells start migrating towards their neighbors. Notably, a directional swarming induction of Paenibacillus cells by neighboring colonies was observed even when the colonies were inoculated on media without glucose. We further show that, when inoculated on plants, the interaction of Paenibacillus swarms with these phytopathogens could have two opposing effects on plant health. The outcome could be either harmful or helpful to the plant, depending on the characteristics of the Paenibacillus species in the swarm. Our results suggest that in mixed populations, interspecies interactions can affect the community's spatial organization and significantly influence disease outcomes in plants.

Reduction of basal levels of (p)ppGpp in experimentally evolved Ralstonia solanacearum as a means of adaptation to plant xylem and legume symbiosis

Nicolas BURKHARDT (1), Mingxing TANG (1), Alice GUIDOT (1), Delphine CAPELA (1)

(1) LIPME, Université de Toulouse, INRAE, CNRS, Castanet-Tolosan, France

Bacteria engage in various interactions with plants, ranging from parasitism to mutualism, on a dynamic continuum shaped by evolutionary pressures. The plant pathogen Ralstonia solanacearum has been used to study evolution along this continuum through a unique combination of two long-term evolution experiments. One experiment investigated its adaptation to novel plant hosts, while the other explored its ability to evolve as a legume symbiont following the horizontal gene transfer of nod-nif-fix genes. Despite the intuitive antagonism between these two adaptive processes, some genes or pathways were convergently mutated in both evolution experiments. Among these convergences, the spoT gene appeared mutated once in one lineage of each experiment. This gene encodes a bidirectional regulator of the (p)ppGpp secondary messenger, a small nucleotide involved in pleiotropic bacterial physiological processes. This work reveals that each mutation confers an enhanced adaptation to both legume symbiosis and xylem colonization for tolerant and susceptible hosts, in a nonspecific manner. The two spoT mutants exhibit pleiotropic phenotypes, notably enhanced metabolism and higher growth rate in synthetic medium. Analysis of the growth of mutants synthesizing various basal quantities of (p)ppGpp suggested a reduction in basal level of (p)ppGpp in the two spoT mutants. This result is reinforced by the discovery that lowered basal (p)ppGpp levels correlate with increased fitness in the xylem of tomato plants. Further characterization of other (p)ppGpp-dependent phenotypes, including pathogenicity, growth, metabolism, motility, stress resistance, and exopolysaccharide production, will allow us to understand how altered (p)ppGpp levels enhance in planta fitness in Ralstonia solanacearum, both for xylem colonization and legume symbiosis.

References:

1. Drew, G. C., Stevens, E. J. & King, K. C. Microbial evolution and transitions along the parasite–mutualist continuum. Nat. Rev. Microbiol. 19, 623–638 (2021)

2. Doin de Moura, G. G., Remigi, P., Masson-Boivin, C. & Capela, D. Experimental Evolution of Legume Symbionts: What Have We Learnt? Genes 11, 339 (2020)

3. Guidot, A. et al. Multihost Experimental Evolution of the Pathogen Ralstonia solanacearum Unveils Genes Involved in Adaptation to Plants. Mol. Biol. Evol. 31, 2913–2928 (2014).

Characterization of the pathogenic interaction between an atypical endophytic bacteria Ensifer adhaerens T4 and the legume Medicago truncatula

Tifaine FOLLETTI (1), Kevin MAGNE (2), Laurent SAUVIAC (1), Chrystel GIBELIN (1), Sophie MASSOT (3), Charlotte TURPIN (3), Marlène DAVANTURE (4), Raphael FORQUET (5), Remy PEYRAUD (5), Fabienne VAILLEAU (1), Pascal RATET (3), Benjamin GOURION (1)

(1) LIPME, Université de Toulouse, INRAE, CNRS, 31326 Castanet-Tolosan, France.

(2) IJPB, INRAE, 78026 Versailles-Saclay, France.

(3) Université Paris-Saclay, Université Paris Cité, CNRS, INRAE, Université Evry, IPS2, 91190 Gif sur Yvette, France.

(4) Université Paris-Saclay, INRAE, CNRS, AgroParisTech, GQE-Le Moulon, 91190, Gif-Sur-Yvette, France.

(5) IMEAN Biotech, 31326 Castanet Tolosan, France.

Rhizobia represent a unique group of bacteria able to establish a symbiotic interaction with plants from Fabaceae family. This mutualistic relationship between legume and nitrogen-fixing bacteria results in the formation of a specialized organ, the nodule, able to fix atmospheric nitrogen and dedicated to host rhizobia. These beneficial interactions lead to a decrease of an expensive use of polluting fertilizer. We isolated from Medicago truncatula nodules such a rhizobia which harbors an atypical behavior : The T4 strain. Whole genome sequencing of this strain reveals that it belongs to the non-symbiotic Ensifer adhaerens group. This bacterium shows contrasted interactions with its host depending on the developmental stage of the plant. It is pathogenic and kills the host when inoculated on germinating seeds but only triggers the formation of non-functional nodules on older seedlings. To our knowledge this kind of bacterium with pathogenic and symbiotic-like behaviors on the same plant species has never been described before and its characterization can lead to better understand the frontier between plant-pathogenic and plant-mutualistic interactions.

Our aim is to characterize the pathogenic behavior of the strain and the molecular mechanisms underlying this interaction. We first determined when the switch occurred between the pathogenic and symbiotic interaction. Using a GFP tagged strain, we characterized the T4 infection process and observed that the strain invades the intercellular space of the cortex to reach and colonize the plant vasculature and then accumulates in cotyledons. T4 inoculated plants keep cotyledons closed and showed no morphological changes at the root level. To determine the molecular mechanisms involved in the pathogenic behavior, we identified T4 candidate genes on the basis of comparative proteomic and BIOLOG data. We constructed mutants altered in these genes and performed pathogenicity assays.

Molecular and physiological consequences of

the Streptomyces sp. GPA1 - Barley relationship

CHEMINAT Margaux, ARSENE-PLOETZE Florence

Institut de Biologie Moléculaire des Plantes, Strasbourg, FRANCE

Plants are in interaction with several microorganisms [1], including bacteria of the Streptomyces genus. Streptomyces are a widely distributed genus of soil bacteria which have several Plant Growth Promoting capacities [2]. Plants select those bacteria in part via their root exudates [3] but the molecular mechanisms involved in this selection aren't well known. We hypothesized that brassinosteroids (BRs), a class of terpenic phytohormones could be involved in barley-Streptomyces selection. We isolated several Streptomyces from barley, among them Streptomyces sp. GPA1 belonging to a new species. This bacterium increased root growth in Wild-type barley (WT) and modified its proteome. We have studied the physiologic and molecular effects of this interaction on both partners and the role of BRs in this relation. For that, we have worked on WT and mutant barley named BW312. This plant had a mutation in the bri1 gene and is deficient in the signaling process mediated by BRs, which is causing an increased production of BRs [4]. Several observations revealed that BRs are involved during GPA1-barley interaction. i) In the presence of the bacterium, the length of WT roots is increased which is not the case for mutant seedlings. ii) Quantitative changes in sterol content of the WT barley were demonstrated, with significantly reduced amounts of stigmasterol, sitosterol and campesterol in the presence of GPA1. This was not the case for BW312 barley. iv) By culturing GPA1 directly in WT or BW312 root exudates, we observed that GPA1 produced biofilm. This biofilm production was different when grown in WT or mutant exudates. Differences in the bacterial proteome were also observed after culture in WT or BW312 exudates, suggesting that the bri1 mutation may have altered the overall composition of exudates. All these data highlighted a new function of BRs in plant-bacteria interaction.

Unravelling the mechanisms of ArcZ sRNA in the virulence control of Dickeya solani

Quentin DUBOIS (1), Typhaine BRUAL (1), Guy CONDEMINE (1), Robert CZAJKOWSKI (2), Agnès RODRIGUE (1), Erwan GUEGUEN (1)

(1) Univ Lyon, Université Claude Bernard Lyon1, CNRS, INSA Lyon, UMR5240 MAP LYON, France

(2) Laboratory of Biologically Active Compounds, Intercollegiate Faculty of Biotechnology of UG and MUG, University of Gdansk, Antoniego Abrahama 58, 80-307 Gdansk, Poland

Dickeya solani is a phytopathogenic bacterium that causes significant agricultural losses, particularly in potato crops. To infect plants, this bacterium produces Plant Cell Wall-Degrading Enzymes (PCWDE). Because the timing of secretion of these enzymes is crucial to avoid triggering the plant's immune system, it is thus tightly regulated. Additionally, motility is essential for host colonization, requiring precise regulation of flagella synthesis at the transcriptional and post-transcriptional levels. In addition to the aforementioned virulence factors, D. solani possesses three polyketide synthase/non-ribosomal peptide synthetase (PKS/NRPS) gene clusters: ooc, zms, and sol, which are responsible for producing oocydin, zeamine, and solanimycin, respectively (1, 2). The sol and zms gene clusters are regulated by ArcZ, a trans-acting non-coding small RNA (sRNA) (2).

ArcZ is a sRNA that requires binding to Hfq and cleavage by RNase E to form a short active processed form. Once active, ArcZ can bind to mRNA targets, regulating them either positively or negatively post-transcriptionally. In Enterobacterales, ArcZ is known for its pleiotropic role, regulating virulence, osmotic stress, acid stress resistance, motility. ArcZ achieves this by binding to numerous mRNAs, including those encoding major regulators such as the sigma factor RpoS of the general stress response and the repressor of virulence PecT (3) .

The objective of this study is to elucidate the role of ArcZ in regulating the virulence of D. solani. To this end, we performed a series of experiments on WT and ΔarcZ D. solani strains, including plant virulence assays, acid stress resistance, motility, PCWDE production and a proteomic analysis comparing proteins produced in ΔarcZ versus WT. Our findings revealed the multifaceted role of ArcZ and its important role in regulating D. solani virulence.

References:

(1) Murphy, A.C., Corney, M., Monson, R.E., Matilla, M.A., Salmond, G.P.C., and Leeper, F.J. (2023) Biosynthesis of Antifungal Solanimycin May Involve an Iterative Nonribosomal Peptide Synthetase Module. ACS Chem Biol 18: 1148–1157.

(2) Brual, T., Effantin, G., Baltenneck, J., Attaiech, L., Grosbois, C., Royer, M., et al. (2023) A natural single nucleotide mutation in the small regulatory RNA ArcZ of Dickeya solani switches off the antimicrobial activities against yeast and bacteria. PLOS Genetics 19: e1010725.

(3) Dubois, Q., Brual, T., Oriol, C., Mandin, P., Condemine, G., and Gueguen, E. (2024) Function and mechanism of action of the small regulatory RNA ArcZ in Enterobacterales. RNA 30: 1107–1121

Investigating Leaf Symbiosis: How do plants cope without their hereditary bacteria?

G. Houdinet, F. Jardinaud, S. Moreau, B. Danneels, A. Carlier

Phyllosym team - LIPME lab - INRAE Occitanie-Toulouse Centre

Some plants host highly specific associations with bacteria in the phyllosphere. These bacteria can be found in specialized structures but remain extracellular, which are sometimes visible on the leaf surface. The evolution of these associations and the mechanisms that sustain their specificity remain poorly understood. Dioscorea sansibarensis is a true yam specie and forms leaf glands at the tip of the leaves which contains a high density of its symbiont, Orrella dioscoreae. This interaction is hereditary and in their natural environment, the symbiont is always present, raising questions about the role of this symbiosis and its specificity. In laboratory conditions, we were able to produce aposymbiotic plants (symbiont-free) that can be re-inoculated by wild-type or genetically modified bacteria. Here, we present data comparing aposymbiotic and symbiotic plants to better understand this leaf symbiosis. Interestingly, plants without symbiont do not have any morphological or phenotypic difference compared to symbiotic plants. This prompted us to investigate further, even if it appeared to have no effect on the plant's overall physiology. We looked at the transcriptomic level. To do so, we also take in account two different organs in the plant: the lamina and the acumen where bacteria gather. Surprisingly, we find no difference at lamina level between symbiotic and aposymbiotic plants, and only a few genes are deregulated at acumen level between these two conditions. These genes seems to be mainly involved in local plant defense in response to the presence of bacteria. In contrast, thousands of genes are differentially regultated in the lamina versus the leaf acumen, suggesting that the leaf gland is indeed a specialized organ. Hence, our main hypothesis is that the acumen is not anymore related to leaf physiology but its main function would be to host and contain their symbiont.

References:

1. Acar et al. ,2022. mBio13:e01033-22.https://doi.org/10.1128/mbio.01033-22

2. Acar et al. PLoS One. 2024 Apr 22;19(4):e0302377. doi: 10.1371/journal.pone.0302377

Role of the long non-coding RNA SYNC1 in transcriptional reprogramming during nodule development

Ibrahim KEITA (1), Arnaud BIASOTTO-CARRERE (1), Sébastien CARRERE (1), Agnès LEPAGE (1), Andreas NIEBEL (1), Matthias BENOIT (1).

(1) Laboratoire des Interactions Plantes Microbes environnement, Auzeville-Tolosane, France

Legumes can form symbiotic root associations with soil bacteria to acquire essential nutrients like nitrogen. In this symbiotic interaction, specialized root organs termed nodules host nitrogen-fixing bacteria, benefiting plant growth1. Nodules form through coordinated symbiotic programs that enable root cell reprogramming and bacterial accommodation1. Transcriptomic studies in Medicago truncatula revealed that thousands of genes are upregulated during nodule development2,3. These symbiotic genes exhibit distinct epigenetic signatures that undergo significant remodeling during the transition from root to nodule3,4. However, the mechanisms driving this transcriptional and epigenetic reprogramming remain unclear. Interestingly, several thousands of long non-coding RNAs (lncRNAs) are transcriptionally activated during nodulation. lncRNAs are emerging as important regulators of gene expression through interactions with transcription factors and chromatin remodeling complexes5. We have identified one lncRNA, named SYNC1, that shows nodule-specific expression and is located in the vicinity of the gene MtRSD, a key symbiosis regulator. MtRSD undergoes dramatic epigenetic changes to unlock its expression in nodules, potentially mediated by SYNC1. We present here data supporting the regulatory role of SYNC1 in the transcriptional and epigenetic reprogramming of MtRSD during nodule formation. Understanding the molecular mechanisms behind nitrogen-fixing symbiosis could lead to novel strategies for promoting sustainable agriculture through enhanced nutrient acquisition in legumes.

References:

1. Oldroyd, G. E. D., Murray, J. D., Poole, P. S. & Downie, J. A. The Rules of Engagement in the Legume-Rhizobial Symbiosis. Annu. Rev. Genet. 45, 119–144 (2011).

2. Roux, B. et al. An integrated analysis of plant and bacterial gene expression in symbiotic root nodules using laser-capture microdissection coupled to RNA sequencing. Plant J. 77, 817–837 (2014).

3. Mergaert, P., Kereszt, A. & Kondorosi, E. Gene Expression in Nitrogen-Fixing Symbiotic Nodule Cells in Medicago truncatula and Other Nodulating Plants. Plant Cell 32, 42–68 (2020).

4. Zanetti ME, Blanco F, Ferrari M, Ariel F, Benoit M, Niebel A, Crespi M. Epigenetic control during root development and symbiosis. Plant Physiol. kiae333 (2024).

5. Rinn, J. L. & Chang, H. Y. Long Noncoding RNAs: Molecular Modalities to Organismal Functions. Annu. Rev. Biochem. 89, 283–308 (2020).

Identification of a bifunctional RNA involved in the virulence of Dickeya dadantii

Sara MOUTACHARRIF (1), Feth-el-Zahar HAICHAR (1), Florence HOMMAIS (1)

(1) Microbiologie, Adaptation et Pathogénie, UMR5240, INSA Lyon, CNRS, Université de Lyon 1, Villeurbanne, France

Dickeya is a genus of enterobacterial plant pathogens that cause soft rot disease in a wide range of plant species, including economically important crops. The infection process consists of two main phases: the asymptomatic phase, during which early virulence factors are produced to colonize plant tissue, and the symptomatic phase, where plant cell wall degrading enzymes macerate the tissue, leading to visible symptoms. Throughout the infection process, D. dadantii encounters environmental changes that require a tight spatial and temporal control of virulence factor production. Numerous transcription factors and nucleoid-associated proteins regulate this process. Additionally, the transcriptional landscape of D. dadantii revealed RNA-mediated regulation as an another layer of control. We found that transcripts of virulence regulators overlap with mRNAs of neighboring genes, and our focus was on the transcriptional repressor PecS. The pecS gene is located adjacent to the argG gene, and the transcriptomic data indicate that their transcripts overlap at the terminator region. We hypothesize that the two transcripts may regulate each other through mRNA-mRNA interaction. Since argG is expressed in the absence of arginine and PecS represses virulence factors, the level of arginine may affect the virulence of D. dadantii. Our results show that the amount of the PecS protein is significantly decreased in the absence of arginine and that it is not due to a regulation of transcription initiation. To assess whether increased production of one transcript can titrate the convergent transcript, we overexpressed one of the transcripts and analyzed the effect on the other transcript by quantifying the protein level. The results suggest that the pecS and argG transcripts are bifunctional RNAs that play the role of both mRNAs and antisense RNAs. Finally, in planta experiments are also carried out to evaluate the impact of apoplastic arginine on the infection process of D. dadantii.

SESSION Evolution, phylogeny, plant and bacterial genomics

Invited speaker: Delphine Capela

Moderators: Delphine Capela, Lionel Gagnevin

Unveiling the evolution of legume symbionts: beyond essential symbiotic gene acquisition

Delphine CAPELA (1), Anne-Claire CAZALE (1), Marvin NAVARRO (1), Saida MOUFFOK (1), Ginaini DOIN DE MOURA (1), Jean KELLER (2), Cyril LIBOUREL (2), Pierre-Marc DELAUX (2), Philippe REMIGI (1)

(1) LIPME, Université de Toulouse, INRAE, CNRS, Castanet-Tolosan, France

(2) Laboratoire de Recherche en Sciences Végétales (LRSV), Université de Toulouse, CNRS, UPS, Toulouse INP, Castanet-Tolosan, France.

To gain insight into the evolutionary mechanisms that have driven the diversification of nitrogen-fixing legume symbionts, we simulated the emergence of novel rhizobia under laboratory conditions. Specifically, we transferred the symbiotic plasmid of Cupriavidus taiwanensis, the symbiont of Mimosa pudica, into Ralstonia solanacearum and subjected this chimera to a series of inoculation-nodulation cycles on M. pudica. These cycles selected for microbial variants with enhanced symbiotic capabilities. In particular, the initial stages of symbiosis, nodulation and nodule cell infection, were rapidly established and significantly improved within the first few cycles. During this adaptive process, the bacteria have accumulated many mutations, among which we identified several highly adaptive ones. Nodulation was acquired through the inactivation of R. solanacearum T3SS, while intracellular infection was achieved and further enhanced by the inactivation or modulation of virulence and metabolic regulators, including HrpG, PhcA, and EfpR. However, despite 60 nodulation cycles, mutualistic nitrogen fixation was not achieved in this experiment. This was partly due to the fact that bacteria do not persist for long in nodule cells. Only low and transient levels of nitrogenase activity were observed in several lineages due to mutations that inactivated the export of putrescine, a polyamine abundantly produced by R. solanacearum. We hypothesize that key genes required for long-term persistence and mutualistic nitrogen fixation, which are present in natural Mimosa symbionts, may be absent in R. solanacearum, thereby preventing the evolution of these traits. Interestingly, we have identified Mimosa-specific genes encoding small secreted proteins whose expression is associated with the intracellular release and persistence of symbionts. Whether these small proteins play a role in the limited persistence of evolved Ralstonia remains to be determined.

References:

1. Libourel C, Keller J, Brichet L, Cazalé AC, Carrère S, Vernié T, Couzigou JM, Callot C, Dufau I, Cauet S, Marande W, Bulach T, Suin A, Masson-Boivin C, Remigi P, Delaux PM, Capela D. 2023. Comparative phylotranscriptomics reveals ancestral and derived root nodule symbiosis programmes. Nat Plants 9:1067-1080.

2. Doin de Moura GG, Mouffok S, Gaudu N, Cazalé AC, Milhes M, Bulach T, Valière S, Roche D, Ferdy JB, Masson-Boivin C, Capela D, Remigi P. 2023. A Selective Bottleneck During Host Entry Drives the Evolution of New Legume Symbionts. Mol Biol Evol 40.

3. Tang M, Bouchez O, Cruveiller S, Masson-Boivin C, Capela D. 2020. Modulation of Quorum Sensing as an Adaptation to Nodule Cell Infection during Experimental Evolution of Legume Symbionts. mBio 11.

4. Capela D, Marchetti M, Clérissi C, Perrier A, Guetta D, Gris C, Valls M, Jauneau A, Cruveiller S, Rocha EPC, Masson-Boivin C. 2017. Recruitment of a Lineage-Specific Virulence Regulatory Pathway Promotes Intracellular Infection by a Plant Pathogen Experimentally Evolved into a Legume Symbiont. Mol Biol Evol 34:2503-2521.

5. Marchetti M, Capela D, Glew M, Cruveiller S, Chane-Woon-Ming B, Gris C, Timmers T, Poinsot V, Gilbert LB, Heeb P, Medigue C, Batut J, Masson-Boivin C. 2010. Experimental Evolution of a Plant Pathogen into a Legume Symbiont. Plos Biology 8.

Analysis of the diversity of prophages in Xylella fastidiosa.

Noe ARROYO-VELEZ (1), Jessica DITTMER (1), Marie-Agnès JACQUES(1)

(1) IRHS, INRAE-Pays de la Loire

Xylella fastidiosa is a gram-negative bacterium of the Xanthomonadace family that colonizes the xylem of a wide range of plant species. It is transmitted by insect vectors that feed on xylem sap and is the causal agent of serious diseases in important crops such as Pierce's disease in grapevine, olive quick decline syndrome, etc. [1]. Xylella fastidiosa originated in the Americas and was subsequently introduced into Europe, where it was first recorded in the Apulia region of Italy in 2013. Since then, it has successfully spread to other European countries, including France, Spain, and Portugal [2]. To date, four subspecies (fastidiosa, multiplex, pauca, and sandyi) have been recorded on the continent, with more than 174 reported hosts [3].The extraordinary adaptability of Xylella fastidiosa to diverse hosts is potentially driven by the high genetic diversity within the species [4]. Multiple studies indicate that horizontal gene transfer through prophage-mediated transformation is a major evolutionary pathway by which bacteria acquire novel traits, enhancing their fitness and expanding their niche [5],[6]. For these reasons, in this work, we used bioinformatics to analyze the repertoire of putative prophages in over 100 genomes of Xylella fastidiosa strains. The analysis revealed a significant number of prophage-derived inserts (with a median of 7), representing a substantial proportion of the bacterial genomes, reaching up to 20% in some strains. Additionally, prophage diversity appeared to be dependent on geographic location, as strains isolated in the same region exhibited similar prophage patterns. Finally, the annotation of the genes encoded in the prophage-inserts revealed their putative roles in various cellular functions, including adaptation under stress conditions which might be relevant for Xylella fastidiosa survival in different host environments.

References:

[1] Sicard A, Zeilinger AR, Vanhove M, Schartel TE, Beal DJ, Daugherty MP, Almeida RPP. Xylella fastidiosa: Insights into an Emerging Plant Pathogen. Annu Rev Phytopathol. 2018 Aug 25;56:181-202. doi: 10.1146/annurev-phyto-080417-045849. Epub 2018 Jun 11. PMID: 29889627.

[2] Serio F, Imbriani G, Girelli CR, Miglietta PP, Scortichini M, Fanizzi FP. A Decade after the Outbreak of Xylella fastidiosa subsp. pauca in Apulia (Southern Italy): Methodical Literature Analysis of Research Strategies. Plants (Basel). 2024 May 22;13(11):1433. doi: 10.3390/plants13111433. PMID: 38891241; PMCID: PMC11175074.

[3] Trkulja V, Tomić A, Iličić R, Nožinić M, Milovanović TP. Xylella fastidiosa in Europe: From the Introduction to the Current Status. Plant Pathol J. 2022 Dec;38(6):551-571. doi: 10.5423/PPJ.RW.09.2022.0127. Epub 2022 Dec 1. PMID: 36503185; PMCID: PMC9742796.

[4] Potnis N, Kandel P, Merfa M, Retchless A, Parker J, Stenger D, Almeida R, Bergsma-Vlami M, Westenberg M, Cobine P, De La Fuente L, Patterns of inter- and intrasubspecific homologous recombination inform eco-evolutionary dynamics of Xylella fastidiosa, The ISME Journal, Volume 13, Issue 9, September 2019, Pages 2319–2333, https://doi.org/10.1038/s41396-019-0423-y

[5] Asadulghani M, Ogura Y, Ooka T, Itoh T, Sawaguchi A, Iguchi A, Nakayama K, Hayashi T. The defective prophage pool of Escherichia coli O157: prophage-prophage interactions potentiate horizontal transfer of virulence determinants. PLoS Pathog. 2009 May;5(5):e1000408. doi: 10.1371/journal.ppat.1000408. Epub 2009 May 1. PMID: 19412337; PMCID: PMC2669165.

[6] Busby, B., Kristensen, D.M. and Koonin, E.V. (2013), Genomics update. Environ Microbiol, 15: 307-312. https://doi.org/10.1111/j.1462-2920.2012.02886.x

A Type 5 integrated prophage of "Candidatus Liberibacter asiaticus", the destructive bacterial pathogens of Citrus Huanglongbing

Frédéric LABBE [1], Claudine BOYER [1], Blandine BENOIST [1], Pierre LEFEUVRE [1], Patrick TURPIN [1], Olivier PRUVOST [1]

(1) CIRAD, UMR PVBMT, F-97410 Saint Pierre, La Réunion, France

Citrus Huanglongbing (HLB) is a bacterial disease that affects citrus trees and is considered the most severe citrus plant disease in the world [1]. Despite their major impact on agronomy, the epidemiology, ecology, and evolution of the three Gram-negative phloem-restricted Candidatus Liberibacter species associated with the disease remain largely unknown due to their non-culturable nature. Among them, "Ca. Liberibacter asiaticus" (CLas) and "Ca. Liberibacter africanus" (CLaf) are the two epidemiologically active species distributed in the citrus-growing regions [2]. These phytopathogenic bacteria are known to have a plastic genome with several phages's genes incorporations which can influence their pathogenicity and adaptability to their host plants and insect vectors [3-5]. The aim of this study was to investigate the integrated prophage-like sequence of CLas by assembling and exploring its first de novo genome assembly from Réunion (V1R1). Two prophages were identified into this 1,271,573 bp single contig de novo assembly, including one prophage that belonged to a new Type 5 CLas prophage (P-V1R1-5) which is highly similar to one prophage previously identified in several CLaf genomes. PCR amplifications specifically targeting this prophage suggested that most CLas strains from Réunion and all CLaf strains from Madagascar and Réunion have incorporated at least parts of this prophage. The identification of this first interspecies variable prophage not only expanded our knowledge of CLas genomic diversity in Réunion, but also provided new insights into the potential role of horizontally transferred elements in the evolution and biology of the sympatric HLB-causing Ca. Liberibacter species.

References:

1. Bové, J. M., Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. Journal of Plant Pathology 2006, 88, 7–37.

2. Gottwald, T. R., Current epidemiological understanding of citrus Huanglongbing. Annual Review of Phytopathology 2010, 48, 119–139.

3. Zhang, S.J.; Flores-Cruz, Z.; Zhou, L.J.; Kang, B.H.; Fleites, L.A.; Gooch, M.D.; Wulff, N.A.; Davis, M.J.; Duan, Y.P.; Gabriel, D.W. "Ca. Liberibacter asiaticus" carries an excision plasmid prophage and a chromosomally integrated prophage that becomes lytic in plant infections. Mol. Plant Microbe Interact 2011, 24, 458–468.

4. Zheng, Z.; Bao, M.L.; Wu, F.N.; Van, H.C.; Chen, J.C.; Deng, X.L. A type 3 prophage of 'Candidatus Liberibacter asiaticus' carrying a restriction-modification system. Phytopathology 2018, 108, 454–461.

5. Dominguez-Mirazo, M.; Jin, R.; Weitz, J.S. Functional and comparative genomic analysis of integrated prophage-like sequences in "Candidatus Liberibacter asiaticus". mSphere 2019, 4, e00409-19.

Evolutionary replacement of T4SS by T6SS for antibacterial killing activity in Xanthomonas

Chloé PEDUZZI (1), Jules BUTCHACAS (2,3), Veronica ROMAN-REYNA (5), François NIKIS (1), Jonathan M. JACOBS (2,3), Ralf KOEBNIK (4) and Claude BRAGARD (1)

(1) Earth & Life Institute, applied microbiology-plant health UCLouvain, Louvain-la-Neuve, Belgium

(2) Department of Plant Pathology, The Ohio State University, Columbus, OH 43210, U.S.A.

(3) Infectious Diseases Institute, The Ohio State University, Columbus, OH 43210, U.S.A.

(4) Plant Health Institute of Montpellier (PHIM), IRD, Montpellier, France

(5) Department of Plant Pathology and Environmental Microbiology, Pennsylvania State University, University Park, PA, USA

Bacteria have evolved diverse antibacterial strategies throughout evolution, enabling them to eliminate competing organisms from their environment. Two distinct contact-dependent antagonistic strategies have been identified within the Xanthomonas genus, the type IV (X-T4SS) and the type VI (T6SS) secretion systems [1,2]. Both systems inject toxic effectors to neighboring bacterial cells, either killing or inhibiting their growth. However, their differing structural origins and distinct toxin repertoires suggest that each system confers fitness advantages to bacteria. Yet it remains unclear why certain bacteria favor one strategy over the other. In this study, the evolution and function of X-T4SS and T6SS in xanthomonads including the Bacterial Leaf Streak pathogen, Xanthomonas translucens (Xt) was investigated. By taking advantage of the natural heterogeneous distribution of both systems in this species and using a combination of genetic and fluorescence-based methods, we demonstrate that X-T4SS and T6SS clade i4 are crucial for inter-bacterial competition in Xt. Antibacterial T6SS-i4 activity was also demonstrated by real-time confocal microscopy imaging. Additionally, comparative genetics and phylogenetic analyses revealed functional replacement of one competition strategy by the other in several xanthomonads lineages. Altogether, these findings expand our current understanding on how closely related bacteria can adapt their competition strategies to survive and succeed in competitive environments.

References:

[1] Zhu, P. C., Li, Y. M., Yang, X., Zou, H. F., Zhu, X. L., Niu, X. N., ... & He, Y. Q. (2020). Type VI secretion system is not required for virulence on rice but for inter-bacterial competition in Xanthomonas oryzae pv. oryzicola. Research in Microbiology, 171(2), 64-73.

[2] Souza, D. P., Oka, G. U., Alvarez-Martinez, C. E., Bisson-Filho, A. W., Dunger, G., Hobeika, L., ... & Farah, C. S. (2015). Bacterial killing via a type IV secretion system. Nature Communications, 6(1), 6453.

Population genomics of Rice Bacterial Leaf Blight in Africa

Ian Lorenzo QUIBOD (1), Florence AUGUY (1), Alexis DEREEPER (1), Mathilde HUTIN (1), Boris SZUREK (1), Sebastien CUNNAC (1), Alvaro PEREZ-QUINTERO (1)

(1) Plant Health Institute of Montpellier, University of Montpellier, CIRAD, INRAE, Institut Agro, IRD, F-34000 Montpellier, France

Crop domestication has a significant effect on the evolutionary trajectory of plant pathogens by providing new ecological niches and abundant resources. The history of cultivated rice in Africa offers an interesting framework to study this, one specie (*Oryza glaberrima*) was domesticated around ~3000 years ago in West Africa, and another (*Oryza sativa*) was introduced around the 16th century by European settlers. Both events likely affected plant-associated microbes. In this study, we used population genomics to understand the diversity and evolution of the bacterial blight pathogen populations in Africa, specifically, *Xanthomonas oryzae* pv. *oryzae* (*AfXoo*). We identified four genetic groups, one recombining and three clonal that seemed to emerge during the introduction of *O. sativa*. We also observed a strict conservation of transcription activator like-effector (TALE) families among isolates, but with some variations in the repeat regions. Transcriptomics revealed either identical or dissimilar targets of different TALE families. Overall, our study provides the evolutionary history and expansion of AfXoo as well as the potential adaptation of TALEs in rice.

Mechanisms governing the interaction between root and bacteria in the model legume L. japonicus

Johan QUILBÉ (1), Troels W. MOURITZEN (1), Francesca TEDESCHI (1), Turgut AKYOL (1), Yusdar MUSTAMIN (2), Masaru BAMBA (2), Shusei SATO (2) and Stig U. ANDERSEN (1)

(1) : Aarhus University, Denmark

(2) : Tohoku University, Japan

Lotus japonicus is a model legume widely used for studying Root Nodule Symbiosis (RNS). Recently, it has also been employed to investigate interactions with more complex microbial communities. By using a synthetic bacterial community (SynCom), we can precisely control bacterial inputs and study host responses at different levels: population-level genomics through GWAS and single-cell expression responses through SingleCell RNAseq. In the GWAS project, we uncovered significant signals leading to the discovery of a novel gene called “ROOMIE1” (ROOT MICROBIOME ESTABLISHMENT 1) and demonstrated the role of microbiomes in shaping plant genome evolution, revealing contrasting signatures between adaptations to climate compared to adaptations to microbes. This highlights the impact of root microbiomes as a selective force in plant natural populations. Conversely, in the SingleCell project, we focused on molecular mechanisms and identified surprising commonalities between RNS pathways and commensal signaling. These findings provide new insights into the overlap between symbiotic and commensal interactions at the single-cell level. Together, these studies deepen our understanding of how Lotus japonicus recruits and interacts with soil bacteria, offering broader implications for plant-microbiome interactions in natural and agricultural settings

References:

GWAS: Quilbé et al., in review at Nature (https://doi.org/10.21203/rs.3.rs-5130034/v1)

Single-Cell: Tedeschi et al., soon to submit.

Genotypic and genomic analyses of French collection of Ralstonia solanacearum strains to improve knowledge of outbreak origins

SALLEN Antinéa (1,2), LECLERC Aurélie (2), PAILLARD Sandrine (1), REIGNAULT Philippe (1), LE ROUX Anne-Claire (2), CUNTY Amandine (1)

(1) Plant Health Laboratory, ANSES, Angers, France

(2) FN3PT/INOV3PT, INRAE-IGEPP, Le Rheu, France

The Ralstonia solanacearum species complex (RSSC), one of the most damaging pests worldwide, includes three distinct species: R. solanacearum (phylotype II), R. pseudosolanacearum (phylotypes I and III) and R. syzygii (phylotype IV). Reports of R. solanacearum have been made in several European countries since 1990 and, more recently, outbreaks of R. pseudosolanacearum as well. Despite all species of the RSSC being classified as quarantine pests in the European Union, few studies have focused on the genotypic diversity of European RSSC strains, and more specifically French ones.

Surveys conducted between 1994 and 2023 in the framework of territory surveillance allowed the collection of more than 360 RSSC strains from mainland France. Phylotype and sequevar characterization revealed that all of them belonged to phylotype II, sequevar 1. In this study, we investigated the genotypic and genomic diversity of those strains, which could help us understand the origin of French outbreaks.

In particular, we explored the potential of Multiple Loci VNTR Analysis (MLVA), widely used to monitor diversity among bacterial populations, to discriminate closely related R. solanacearum strains. A novel MLVA scheme was therefore specifically designed for French R. solanacearum strains. Nearly 30 different VNTR profiles were discriminated among the near-clonal French strains and were analyzed in relation to isolation year, host, etc. In order to estimate the diversity of French strains at the European level, 29 European strains of R. solanacearum phylotype II, sequevar 1 were included in the MLVA study as well.

In addition, the genomes of 235 French R. solanacearum strains were sequenced to further investigate their genomic diversity. Previously available worlwide public genomes of R. solanacearum were considered as well. These genomic data complement the genotypic ones obtained by MLVA and provide evidence for the origins and dates of introduction of R. solanacearum in mainland France.

The evolution of evolvability in *Xanthomonas*

Alvaro L PEREZ-QUINTERO (1), Pablo BERTOGNA (1), Ian Lorenzo QUIBOD (1), Boris SZUREK (1)

(1) Plant Health Institute of Montpellier, University of Montpellier, CIRAD, INRAE, Institut Agro, IRD, F-34000 Montpellier, France

All living beings evolve but they don't do so at the same speed. The "evolvability" of an organism, this is, their capacity to generate and tolerate/select variation, is a trait that can be coded in the genome and can be selected for. Certain genomic features including the presence of mobile elements and repetitive regions can accelerate the speed at which variants appear in a population; under highly variable environments having a more evolvable genome can be advantageous and will thus be selected for. We propose this is the case for arms-races between certain *Xanthomonas* species and their plant hosts, where pressure given by resistance in the plant has lead to highly evolvable bacterial genomes. Furthermore, particular genes like the repeat-containing TAL effectors, might have been selected in certain groups due to their high evolvability. TAL effectors, as a result of their repetitive character, are prone to recombinaton, which leads to variation in repeat number and order, which allows diversification of virulence functions and evasion of recognition by the host. I will present examples and hypotheses on how TALEs evolve in populations, and how in general, high rates of recombination between repetitive regions is a feature that has been selected for high evolvability in *Xanthomonas.*

SESSION Chemical ecology, secondary metabolites

Invited speaker: Laure Weisskopf

Moderators (Thursday 16th): Laure Weisskopf, Florence Wisniewski-Dyé

Moderators (Friday 17th): Florence Hommais, Benoit Alunni

Chemical communication and its impact on the expression of biocontrol traits in plant-associated bacteria

Sébastien BRUISSON, Abhishek ANAND, Livia JERJEN, Floriane L'HARIDON, Ola ABDELRAHMAN and Laure WEISSKOPF

Department of Biology, University of Fribourg, Chemin du Musée 10, CH-1700 Fribourg, Switzerland

Plants are colonized by a diverse microflora both at the root and at the shoot level. This contributes to plant health, e.g. by secreting antimicrobial compounds, by inducing the plant immune defenses or by depriving pathogens of important resources such as iron. We are interested in understanding how the microbiome contributes to plant health and use crops of agronomical relevance such as potato and grapevine to answer this question. We have recently discovered that plant-associated bacteria and fungi can detect the presence of competing microbes via their volatile emission and react by upregulating antimicrobial volatile emission and/or siderophore production. In turn, some of the volatile chemical signals emitted by beneficial root-associated Pseudomonas can remotely influence the behavior of other rhizosphere inhabitants, leading to diminished siderophore production, reduced motility and increased biofilm formation. These modulated traits are of relevance for both the ability of the microbes to successfully colonize their host plant and to competitively inhibit plant pathogens. These new findings lift the veil on the complex chemical communication taking place within the plant microbiota, which can be mediated by both volatile and non-volatile signaling compounds. Understanding the basis of this communication and identifying the chemical signals leading to up- or downregulation of biocontrol-relevant traits such as siderophore production or emission of antimicrobial volatiles in plant-associated bacteria will open significant avenues for improved microbe-mediated crop protection in the future.

References:

Anand, A, Falquet, L, Abou-Mansour, E, L'Haridon, F, Keel, C & Weisskopf, L. Biological hydrogen cyanide emission globally impacts the physiology of both HCN-emitting and HCN-perceiving Pseudomonas. MBio 14, (2023).

Bruisson, S, Alfiky, A, L'Haridon, F & Weisskopf, L. A new system to study directional volatile-mediated interactions reveals the ability of fungi to specifically react to other fungal volatiles. Front. Ecol. Evol. 11, (2023).

Weisskopf, L. Microbes to the Rescue – Exploring the Chemistry of Microbial Communication and Using it to Protect Plant Health. Chimia (Aarau). 76, 939–944 (2022).

Weisskopf, L, Schulz, S & Garbeva, P. Microbial volatile organic compounds in intra-kingdom and inter-kingdom interactions. Nat. Rev. Microbiol. 19, 391–404 (2021).

Phytobacteria extracellular vesicles as understimated actors in plant-bacteria interactions

Timothée ZANNIS-PEYROT (1), Lucas DEGUSSEAU (1), Pierre-Yves DUGAS (2), Fabiola BASTIAN (3), Matthieu GAUCHER (4), Vincent GAILLARD (1), Florence WISNIEWSKI-DYE (1), Isabelle KERZAON (1), Céline LAVIRE (1)(*), Ludovic VIAL (1)(*)

(1) Université Claude Bernard Lyon 1, UMR 5557 Ecologie Microbienne, CNRS, INRAE, VetAgro Sup, UCBL, F-69622, Villeurbanne, Lyon, France

(2) Université Claude Bernard Lyon 1, CNRS, CPE Lyon, UMR 5128, Laboratoire Catalyse, Polymérisation, Procédés et Matériaux (CP2M), 69616 Villeurbanne, France

(3) DTAMB, FR BioEEnVis, Université Claude Bernard Lyon 1, Lyon, France

(4) IRHS-UMR1345, Université d'Angers, INRAE, Institut Agro, SFR 4207 QuaSaV, F-49071, Beaucouzé, France

* Both authors participated equally to this work

Bacterial extracellular vesicles (BEVs) are lipidic shuttles that facilitate the export of cellular materials over long distances from the cell. BEVs can transport lipids, proteins, nucleic acids, and metabolites. They play roles in virulence, inter-species competition, and the induction of the host immune response. While they have primarily been investigated in animal-bacteria interactions, knowledge regarding phytobacterial BEVs remains limited. Recent findings revealed that various biotic factors can regulate BEVs production, as demonstrated in Pseudomonas putida where lignin derivatives influence BEVs size and cargo (1). Hydroxycinnamic acids, such as ferulic acid, are lignin components abundantly released in the plant environment, where they impact the ecology of numerous phytobacteria. Azospirillum sp. B510, a phytobeneficial bacteria, induces the accumulation of hydroxycinnamic acids derivatives in the plant and can use them as carbon sources (2). We hypothesized that the presence of ferulic acid (FA) in the environment of Azospirillum sp. B510 would influence its BEVs production in terms of size, quantity, and cargo. Conversely, we also proposed that BEVs from this phytobacterium would influence plant metabolites and enhance protection against pathogens. We assessed the effect of FA on the BEVs production by Azospirillum sp. B510 using electron microscopy and NTA. Through LC-MS² analyses, we characterized the influence of FA on the composition of BEVs cargo. Finally, after an exposure to BEVs, we compared Solanum lycopersicum specialized metabolite profiles and the defense gene expression. Our results indicate that FA (plant environment) affects the production of BEVs by Azospirillum sp. B510 and its BEVs also impacts plant physiology depending on their cargos. This research provides the first evidence of a global effect of BEVs on the plant and highlights the dynamic nature of plant-bacteria interactions mediated by BEVs.

References:

(1) Salvachúa, D., Werner, A.Z., Pardo, I., Michalska, M., Black, B.A., Donohoe, B.S., et al. (2020) Outer membrane vesicles catabolize lignin-derived aromatic compounds in Pseudomonas putida KT2440. Proc Natl Acad Sci U S A 117: 9302–9310.

(2) Valette, M., Rey, M., Gerin, F., Comte, G., and Wisniewski-Dyé, F. (2020) A common metabolomic signature is observed upon inoculation of rice roots with various rhizobacteria. J Integr Plant Biol 62: 228–246.

Ecology and mineral weathering ability of the ectomycorrhizosphere strain Pseudomonas sp. PML3(3)

Ségolène BOUCHE (1,2), Marie-Pierre TURPAULT (2), Stéphane UROZ (1,2)

(1) IAM, UMR1136 INRAE Université de Lorraine, 54280 Champenoux, France

(2) BEF, UR1138 INRAE, 54280 Champenoux, France

The forests of temperate regions are often developed on acidic and rocky soils characterized by low nutrient availability. To cope with such conditions trees have developed associations in their root system (the mycorrhizosphere) with symbiotic fungi and bacteria. Through their ability to weather minerals and transfer nutrients, these microorganisms participate to the nutrition and health of trees. Noticeably, the enrichment of effective mineral weathering (MWe) bacteria is considered to be linked to the carbon sources contained in fungal and tree root exudates. In such acidic soil, culturable representatives of the genus Pseudomonas are quite rare and only few strains have been isolated from the mycorrhizosphere and analyzed for their MWe ability. The characterization of the molecular mechanisms and genes involved remains to be deepened regarding this genus. To do it, we considered the model strain Pseudomonas sp. PML3(3) isolated from the oak- Scleroderma citrinum ectomycorrhizosphere.

The objectives were i) to characterize the functional properties of the model strain ii) to assess its MWe ability and the genes and/or clusters potentially involved, and iii) to study the effect of the type of carbon source consumed. To achieve this, the genome of the strain PML3(3) was sequenced, assembled and analyzed to identify genes of interest and particularly those related to mineral weathering. Moreover, MWe assays were conducted using different biotests and culture conditions to determine the relative effect of 11 carbon sources, often found in root exudates. Our first results revealed that the genome of strain PML3(3) contains genes encoding the canonical GDH-PQQ system and two clusters responsible for siderophores production. The bioassays performed confirmed that PML3(3) produces at least one siderophore and can strongly acidify the solution with different carbon substrates. The weathering experiments highlighted that strain PML3(3) is effective at weathering biotite.

Bacteriocins of the Ralstonia solacearum species complex:

Biochemical and genetic characterization

Eva CALY SIMBOU (1;2), Stéphane RAMIN (1) , Jean-Jacques CHERON (2), Stéphanie JAVEGNY (2), Stéphane POUSSIER (1) ; Yann PECRIX (2)

(1) UMR PVBMT, Université de La Réunion, Saint-Pierre, France.

(2) UMR PVBMT, CIRAD, Saint-Pierre, France.

Bacteriocins are antimicrobial peptides produced by numerous bacterial species, and generally have a narrow spectrum of inhibition (targeting closely related strains or species).1. Already used in various fields, including agri-food, health and agriculture 2, bacteriocins are intended to constitute an alternative to antibiotics and an effective strategy to control multidrug-resistant bacteria.

The phytopathogenic bacteria belonging to the Ralstonia solanacearum species complex (Rssc) are responsible for bacterial wilt, the second most damaging phytobacterial disease in the world, reported on every continent, with a high prevalence in tropical and subtropical regions 5. By obstructing the xylem of host plants, Rssc strains can induce irreversible wilting in over 450 plant species, including important hosts such as bananas, cassava, potato, tomato6.

The Rssc is classified into four phylotypes reflecting their geographical origin : Asia (Phylotype I), America (II), Africa (III) or Indonesia (IV). These phylotypes are further classified into around 70 sequevars based on the endoglucanase (egl) partial gene sequence. 6. In the south-western Indian ocean (SWIO), strains of phylotype I sequevar 31 (I-31) predominates in small islands (Reunion, Mayotte, Comoros, Seychelles), whereas in Madagascar, I-18 strains are mainly found.. Although no bacteriocins have yet been characterized in Rssc, we have recently shown that these two epidemic lines display bacteriocin-like antibacterial activity targeting less prevalent strains in the SWIO. This result, which underlines the importance of bacteriocins in intraspecific competitions, also offers new perspectives in the development of innovative biocontrol tools. In this context, we have set up a pipeline to biochemically characterize these bacteriocins and evaluate their use to control bacterial wilt in solanaceous crops.

References:

1. Jiong Zou, Han Jiang, Hui Cheng, Jiehong Fang, Guangrong Huanf. Strategies for screening, purification and characterization of bacteriocins. (2018) doi:10.1016/j.ijbiomac.2018.05.233.

2. Kim, J.-G. et al. Bases of biocontrol: Sequence predicts synthesis and mode of action of agrocin 84, the Trojan Horse antibiotic that controls crown gall. Proc. Natl. Acad. Sci. 103, 8846–8851 (2006).

3. Rasoamanana, H. N. A. Biologie des populations du complexe d'espèces Ralstonia solanacearum à Madagascar et dans le sud-ouest de l'océan indien. (Université de la Réunion, 2022).

4. Mansfield, J. et al. Top 10 plant pathogenic bacteria in molecular plant pathology: Top 10 plant pathogenic bacteria. Mol. Plant Pathol. 13, 614–629 (2012).

5. Álvarez, B., Biosca, E. G. & López, M. M. On the life of Ralstonia solanacearum, a destructive bacterial plant pathogen. 14 (2010).

6. Peeters, N., Guidot, A., Vailleau, F. & Valls, M. Ralstonia solanacearum , a widespread bacterial plant pathogen in the post-genomic era: Ralstonia solanacearum and bacterial wilt disease. Mol. Plant Pathol. 14, 651–662 (2013).

Competitive colonization of the potato host by the phytopathogen Dickeya solani

Coline AMARO-LAUER (1) , Valentin LEROUX (2), Raudeine HASSIAM (2), Erwan GUEGUEN (3), Véronique EPARVIER (2), Denis FAURE (1)

(1) Plant- Bacteria Interactions team Institut de Biologie Integrative de la Cellule (I2BC)

(2) Functional Chemistry-Chemical Ecology team, Institut de Chimie des Substances Naturelles (ICSN)

(3) Equipe Trafic et Signalisation Membranaires chez les Bactéries, Laboratoire MAP, INSA Lyon

In emergence in Europe since the 2000's, Dickeya solani is a pectinolytic bacterium targeting the potato plant. D. solani induces the blackleg and soft-rot diseases on potato stems and tubers, diseases representing millions of euros of loss per year for the potato plant sector. Explorations of D. solani genome have shown the presence of NRPS/PKS gene clusters coding for antimicrobial metabolites [1,2]. These metabolites could play a major role in the competition against the resident microbiota, especially in a context of an emergent pathogen colonizing a new host. This research project aims to better understand the role of these antimicrobial metabolites in the colonization of the potato plant by D. solani. To evaluate the impact of the production of antimicrobial metabolites, deletion mutants for each biosynthesis pathway have been constructed. Their activity has been compared to the wild type strain. Thanks to a mutant/wild type screening, sensitive microbial targets for each metabolite were identified in a collection composed of potato-specific pathogens, soil isolates and biocontrol agents. These growth inhibition tests supplemented with chemical analysis demonstrated the mutants impairments in their ability to produce the corresponding metabolite and to inhibit growth of other microbes. Inoculation assays in planta were also performed with all strains on potato plants and shown impairments in the mutants ability to colonize the host and to induce symptoms. Metabarcoding analyses are on-going and we expect differences in root microbiota diversity between mutants and wild type conditions. Moreover, metabolomic and transcriptomic assays in presence of sensitive targets and of D. solani are planned to understand the interplay occurring during the colonization of the potato host between pathogen and resident root microbiota. Overall, this work revealed the importance of the production of secondary metabolites in a plant-microbiota-pathogen ecological system.

References:

[1] Robic, K., Munier, E., Effantin, G., Lachat, J., Naquin, D., Gueguen, E., and Faure, D. (2023). Dissimilar gene repertoires of Dickeya solani involved in the colonization of lesions and roots of Solanum tuberosum. Front. Plant Sci. 14, 1154110. 10.3389/fpls.2023.1154110.

[2] Brual, T., Effantin, G., Baltenneck, J., Attaiech, L., Grosbois, C., Royer, M., Cigna, J., Faure, D., Hugouvieux- Cotte-Pattat, N., and Gueguen, E. (2023). A natural single nucleotide mutation in the small regulatory RNA ArcZ of Dickeya solani switches off the antimicrobial activities against yeast and bacteria. PLOS Genet. 19, e1010725. 10.1371/ journal.pgen.1010725.

Roles of salicylates in the regulation of poplar microbiome colonization: from defence signalling molecule to carbon source.

A. DEVEAU (1), F. FRACCHIA (1), C. KAMMERER (1), M. LEVASSEUR (1,2), V. CARRE (2), D. PELLETIER (3), N. L. ENGLE (3), T. J. TSCHAPLINSKY (3), C. VENEAULT-FOURREY (1)

(1) Université de Lorraine, INRAE, IAM, Nancy, France

(2) Université de Lorraine LCPA2MC, Metz, France

(3) Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, USA

The initial assembly of the tree microbiota from root to shoot, and its link to root exudates and tissue metabolites, is not fully understood. Poplar serves as a model for studying these processes, as it can be clonally propagated in sterile conditions and genetically modified. Using a mesocosm device in which sterile poplar cuttings can be cultivated on sterile gamma irradiated or microbially colonized soils, we demonstrated by combining metabarcoding and confocal microscopy approaches that root microbial colonisation exhibited a dynamic response, initially involving saprophytic microorganisms and later transitioning to endophytes and symbionts (1,2). We further characterised how fungal and bacterial communities are altering root exudates as well as root and shoot metabolomes by gas chromatography-mass spectrometry. Microbial colonisation triggered rapid and substantial alterations in both the composition and quantity of root exudates, with over 70 metabolites exclusively identified in remarkably high abundances in the absence of microorganisms, including defence compounds of the salicylate family. Those later were strongly depleted in the roots exudates in the presence of microorganisms, suggesting the rewiring of root metabolism and/or a metabolization by the microorganisms. On this basis, we hypothesized that salicylates play a role in structuring of the colonization of poplar roots by microorganisms and that some saprophytic microorganisms which are early colonizers of the rhizosphere could metabolize salicylates and by doing so allow the growth of sensitive late comers. To test this hypothesis, we measured the sensitivity of a collection of bacteria and of fungi isolated from the rhizosphere of poplars and we tested their ability to metabolize salicylates. Results indicate that these abilities differ depending on compounds and microbial strains suggesting that different salicylates play distinct roles in shaping poplar microbial communities.

References:

1 - Fracchia, F., Guinet, F., Engle, N.L. et al. Microbial colonisation rewires the composition and content of poplar root exudates, root and shoot metabolomes. Microbiome 2024, 12, 173.

2 - Fracchia,F., Mangeot-Peter, L., Jacquot,L., Martin,F., Veneault-Fourrey,C., Deveau, A. Colonization of Naive Roots from Populus tremula × alba Involves Successive Waves of Fungi and Bacteria with Different Trophic Abilities. Applied and Environmental Microbiology, 2021, 87 (6).

Antifungal and plant growth promoting activity of avocado phyllosphere bacteria and their organic extracts

Nayeli A. DIYARZA-SANDOVAL (1), Alfonso MÉNDEZ-BRAVO (2,3), Juan L. MONRIBOT-VILLANUEVA (4), José A. GUERRERO-ANALCO (4), Edgardo SEPÚLVEDA (5), Frédérique REVERCHON (1,3)

(1) Red de Diversidad Biológica del Occidente Mexicano, Centro Regional del Bajío, Instituto de Ecología, A.C., Mexico

(2) CONAHCYT - Escuela Nacional de Estudios Superiores Unidad-Morelia, Universidad Autónoma de México, Mexico

(3) UMR Interactions Arbres-Microorganismes, INRAE Grand-Est Nancy, France

(4) Red de Estudios Moleculares Avanzados, Instituto de Ecología, A.C., Mexico

(5) CONAHCYT - Departamento de Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada, Mexico

Crop-associated bacteria represent a promising source of microorganisms with biotechnological potential. However, most research has been directed at rhizosphere bacteria and the beneficial properties of phyllosphere bacteria have been largely overlooked. Here, we investigated the antifungal and plant growth-promoting properties of avocado phyllosphere bacteria and their organic extracts. In vitro assays were performed to assess the antagonistic activity of phyllosphere bacteria against two avocado pathogens, Fusarium sp. and Colletotrichum gloeosporioides, leading to the selection of 10 bacterial strains identified as belonging to the genera Pandoraea, Kocuria, Robertmurraya, Calidifontibacillus, Bacillus and Erwinia. These bacteria were then tested in vitro for plant growth-promoting traits such as biofilm formation, nutrient solubilization, production of indole acetic-acid and siderophores, and stimulation of Arabidopsis thaliana development. In particular, four strains were able to enhance the biomass and lateral root growth of A. thaliana, most likely through an activation of the auxin pathway. The obtained results allowed the selection of two strains, Robertmurraya sp. B4 and Pandoraea B1, for the obtention of bacterial organic extracts (EtOAc and n-BuOH) and subsequent chemical profiling through UPLC–HRMS. Bacterial organic extracts displayed antifungal activity against the two fungal pathogens, the strongest inhibition being induced by Robertmurraya n-BuOH extract. Tentative identification of distintive compounds showed the presence of metabolites involved in tryptophan and biotin metabolism and in antibiotic secretion, which sheds a light on the possible mechanisms underlying the beneficial properties displayed by these phyllosphere strains.

SESSION Effect of the environment, Epidemiology, Ecology

Invited speaker: Edward Topp

Moderators: Edward Topp, Marie-Anne Barny

The development and transmission of antibiotic resistance in crop production systems.

Edward Topp

Agroecology Research Unit, INRAE, University of Burgundy, 21000 Dijon, France.

A common and important farming practice is to fertilize crop ground with fecal material of animal or human origin. In many parts of the world now the use of reclaimed sewage effluent for irrigation is becoming an increasingly important practice as precipitation declines due to climate change. Generally, not in Europe, but elsewhere antibiotics are used as pesticides to control bacterial crop diseases. These practices entrain antibiotic-resistant bacteria, antibiotics, and other potentially co-selective chemicals into crop ground. This presentation will discuss what is known and some knowledge gaps regarding antibiotic-resistance in these systems, the potential for contamination of crops and potential risk to human health.

Exploring the xylem-sap to unravel interactions between bacterial xylem endophytes and two phytoathogenic bacteria, Xylella fastidiosa Brenneria salicis in Salicaceae through metagenomics and in vitro studies.

Lena Pesenti (1), Claude Bragard (1)

(1) Université catholique de Louvain, Earth and Life Institute, Applied Microbiology, Plant Health Lab - Louvain-la-Neuve, Belgique

Xylella fastidiosa and Brenneria salicis, two xylem-specialist phytopathogenic bacteria, often cause severe decline in their host plants. However, some infected plants show no symptoms, and the factors behind this dual symptomatology remain unknown (1,2). Recent studies suggest that interactions between pathogenic bacteria and the endophytic microbiota within the xylem could play a role. Endophytes are known to contribute to plant health, but the protection mechanisms are still not fully understood (3). This project explores how endophytes interfere with the colonization of phytopathogenic bacteria in the xylem. Based on the knowledge of interactions between Salicaceae plants and both pathogens, more than 350 bacterial endophytes were isolated, with several identified as potential biocontrol agents. Simple confrontation assays were used to study their interactions with X. fastidiosa KLN59.3 and B. salicis LMG2698. Endophytes that inhibited one or both pathogens were further analyzed for traits like siderophore and indole production, phosphate solubilization, motility, and biofilm formation. Genome of 15 promising strains (Bacillus velezensis, B. subtilis, B. pseudomycoides, B. mycoides, B. cereus, Pseudomonas graminis, P. coleopterorum, Erwinia rhapontici) were sequenced and are/will be transformed to express fluorescent protein. This allows tracking their growth, propagation, and interaction with the pathogens in xylem vessels, using both Salicaceae hosts and Nicotiana tabacum as test species.

References:

(1)Janse, J. D., & Obradovic, A. (2010). Xylella fastidiosa: Its biology, diagnosis, control and risks. Journal of Plant Pathology, 92(1 SUPPL.).

(2)Maes, M., Huvenne, H., & Messens, E. (2009). Brenneria salicis, the bacterium causing watermark disease in willow, resides as an endophyte in wood. Environmental Microbiology, 11(6), 1453–1462. https://doi.org/10.1111/j.1462-2920.2009.01873.x

(3)Lengrand, S., Pesenti, L., Bragard, C., & Legrève, A. (2024). Bacterial endophytome sources , profile and dynamics — a conceptual framework. Frontiers in Sustainable Food Systems, 8(March), 1–16. https://doi.org/10.3389/fsufs.2024.1378436

Impact of an introduced species of alder (Alnus cordata) on the associated Frankia diversity and nitrogen cycling microbial communities

Gwennaëlle HENRY (1), Thomas KREMER (1), Charline CREUZE DES CHATELIERS (1), Abigaïl DELORT (1), Pascale FOURNIER (1), Jonathan GERVAIX (1), Alessandro FLORIO (1), Aude HERRERA-BELAROUSSI (1), Amélie CANTAREL (1), Sandra KIM TIAM (1)

(1) UMR 5557, Laboratoire d'Ecologie Microbienne, Université Claude Bernard Lyon 1, 43 Boulevard du 11 Novembre, 69622 Villeurbanne CEDEX, France

The Alnus genus, consisting of actinorhizal trees, can establish a nitrogen-fixing symbiosis with Frankia, a genus of soil gram-positive actinobacteria able to convert atmospheric nitrogen into ammonia. This symbiosis allows Alnus species to thrive in nitrogen depleted environments and act as pioneer species(1). The Dombes region is a continental wetland in which rainfall decrease has been observed in recent years. With climate change, there is a risk of increased drought periods that would affect general water supply of the ecosystem(2). The indigenous alder tree species Alnus glutinosa is dependent on water supply and contributes to atmospheric nitrogen entry in the N-cycle via its symbiosis with Frankia. Alnus cordata, a Mediterranean alder species known to be more drought resistant(3), was introduced in the Dombes and lives sympatrically with Alnus glutinosa. If A. glutinosa population decreases due to climate change, using A. cordata as a replacement could be considered, provided it could occupy the same ecological niche. We assessed the impact of A. cordata introduction on soil nitrogen content and N-cycle related enzyme activities. We also compared Frankia strains associated with roots of both alder species and nitrogen fixation activities within symbiotic root nodules. Results showed that A. cordata did not modify accumulation of soil organic matter, organic carbon and nitrogen in comparison with A. glutinosa and that microbial enzymatic activities were identical under both species. Both alder species were colonized by two distinct phylogenetic clades of Frankia strains and nodule N-fixing activities did not differ between species. Looking into abundances and diversity of N-cycle microbial communities is the next step to assess the effect of A. cordata introduction. Ongoing analysis of qPCR and sequencing data from rhizospheric soils of both alder species will contribute to further evaluate if A. cordata could occupy the same ecological niche as A. glutinosa

References:

(1) Pawlowski, K. and Sirrenberg, A. (2003) Symbiosis between Frankia and actinorhizal plants: root nodules of non-legumes. Indian J Exp Biol. 41, 1165–1183.

(2) Carroget, A., Perrin, C., Sauquet, E., Vidal, J.-P., Chazot, S., Chauveau, M. and ROUCHY, N. (2017) Explore 2070 : quelle utilisation d'un exercice prospectif sur les impacts des changements climatiques à l'échelle nationale pour définir des stratégies d'adaptation ? Sciences Eaux & Territoires. 4–11.

(3) Hall, R.B. and Burgess, D. (1990) Evaluation of Alnus species and hybrids. Biomass, Forestry, Forest Biomass, and Biomass Conversion: The IEA Bioenergy Agreement (1986-1989) Summary Reports. 22, 21–34.

Deciphering population dynamics of Ralstonia solanacearum inside a host tomato

Sylvain VICENTE (1), Isabelle MILA (1), Stephane GENIN (1), Nemo PEETERS (1)

Collaborators: Guilhem REYT (1), Lionel ROQUES (2), Yann PECRIX (3)

(1) LIPME, INRAE/CNRS, Toulouse

(2) BIOSP, INRAE, Avignon

(3) PVBMT, CIRAD, Saint-Pierre (La Réunion)

Bacterial wilt is a devastating plant disease that affects globally important crops such as tomatoes, potatoes, and bananas. The disease is caused by Ralstonia solanacearum, a soil-borne phytopathogen. This bacterium infects the roots of susceptible plants and rapidly colonises the xylem, leading to vascular occlusion, wilting, and eventually plant death. While significant research has been dedicated to understanding bacterial virulence and plant immune responses, a quantitative understanding of the infection cycle remains lacking.

In this presentation, I will introduce a novel approach that integrates microbiology, ecology, population genetics, and mathematical modelling to track pathogen population dynamics at the level of individual plant compartments. We employ a method called STAMP (Sequence Tag-base Analysis of Microbial Population), in which plants are infected with populations of 120 isogenic R. solanacearum strains, each tagged with a unique artificial genetic marker at a neutral locus. By comparing the loss of diversity in the pathogen population within plant tissues to that in the initial inoculum, we can identify population bottlenecks imposed by plant defences during infection.

I will describe how we combine Nanopore next-generation sequencing with a mathematical framework designed to analyse neutral diversity, addressing the following question: What are the spatio-temporal population dynamics of R. solanacearum in susceptible versus tolerant tomato plants? This presentation will highlight our latest results on quantifying the physical and immune barriers that influence infection success in both susceptible and tolerant plant genotypes under varying infection conditions. In particular, I will discuss several key metrics (including infection founding population size, subpopulation identification, and genetic diversity analysis) that offer a deeper understanding of how pathogenic bacteria infect and spread within plants.

References:

Abel, S., Wiesch, P.A. zur, Chang, H.-H., Davis, B.M., Lipsitch, M., Waldor, M.K., 2015. STAMP: Sequence tag-based analysis of microbial population dynamics. Nat. Methods 12, 223. https://doi.org/10.1038/nmeth.3253

Bacterial Leaf Blight of rice : an emerging threat to rice cultivation in East Africa

Ravo RABEKIJANA(1,2), Harinjaka RAVELOSON H (3), Alexis DEREEPER (2), Camille BLASCO (2), Laurent BROTTIER (2), Natolotra RAKOTONANAHARY (3), Ibrahim HASHIM (4)., Happy LEONARD (4), Reube MIHAYO (4), Everlyne NGANGA (5), Joseph BIGIRIMANA (6), Maureen EYOKIA (6), Geoffrey ONAGA G7, Claire NEEMA (2), Sebastien CUNNAC (2), Florence AUGUY (2), Boris SZUREK (2), Mathilde HUTIN (2).

(1) Université d'Antananarivo, École Supérieure des Sciences Agronomiques (ESSA), Antananarivo, Madagascar

(2) IRD, Institut de Recherche pour le Développement UMR PHIM, Plant Health Institute of Montpellier Univ Montpellier, CIRAD, INRAE, Institut Agro, IRD, Montpellier, France

(3) Centre Régional de Recherche du FOFIFA d'Antsirabe, Antsirabe, Madagascar

(4) TARI, Ifakara Station, Tanzania

(5) International Rice Research Institute (IRRI) - Africa Regional Office, P.O. Box 30709 Nairobi 00100, Kenya.

(6) International Rice Research Institute-Burundi Office, Mutanga Campus of Burundi University, Avenue de l'UNESCO nr 2, Bujumbura-Burundi

(7) Africa Rice Center (AfricaRice) 01 BP 2551 Bouake 01, Cote d'Ivoire

Xanthomonas oryzae pv. oryzae (Xoo), the causal agent of Bacterial Leaf Blight (BLB) of rice, leads to yield losses of up to 70% and threatens rice production. In 2019, we reported the first identification of BLB in Madagascar, a country where 90% of agricultural activity relies on rice production, and the emergence of the disease in Tanzania, one of the largest rice-producing countries in East Africa. Since then, annual surveys have shown a rapid and worrying spread of BLB in these countries, as well as in others (e.g. Kenya and Uganda), leading to the establishment of a collection of over 450 Xoo strains. Microsatellite genotyping of these strains revealed that they all belong to the same clonal complex in Madagascar and to another in continental East Africa. This finding suggests that Xoo was introduced to these two countries independently and recently. Moreover, our data indicate that pathogen haplotypes are maintained across farming seasons and that new haplotypes emerge annually. This suggests that the pathogen population has diversified over the past five years. Whole genome sequencing of 9 strains from these two countries indicates a possible introduction of Xoo from two different Asian countries. The recent introduction of Xoo in Madagascar, and the rapid spread of a few haplotypes belonging to the same clonal complex strongly suggest a role for infected seeds in BLB epidemiology. This is also supported by the molecular epidemiology study that we have conducted in Tanzania, Uganda and Kenya. We have also genotyped strains collected from different weed species growing in or around rice fields to better understand their role as reservoirs. Understanding disease epidemiology is critical to preventing new introductions, and reducing pathogen transmission is key to controlling disease spread and limiting its impact in the absence of resistant host varieties.

SESSION Plant immunity & Plant Health management: diagnostic, biocontrol

Invited speaker: Guillaume Chesneau

Moderators: Guillaume Chesneau, Barbara Pivato

The root microbiota: unraveling microbial multi-kingdom metabolic interactions and their implications for plant health

Guillaume Chesneau, Stéphane Hacquard

Max Planck Institute for Plant Breeding Research, Department of Plant Microbe Interactions, 50829, Cologne, Germany

Corresponding authors : gchesneau@mpipz.mpg.de

In the Multitrophic Plant-Microbe Interactions group, we investigate the mechanisms governing microbial communities in plant roots. By integrating microbial community profiling from *Arabidopsis thaliana* with culture collections and gnotobiotic plant systems, we explore how host-microbe and microbe-microbe interactions shape root microbiota and influence plant health. In recent years, our extensive genomic analyses have identified key multi-kingdom genetic factors driving microbial root colonization, including fungal PL1_7 CAZymes and bacterial genes such as typA, pstABCS, and exbD1, alongside plant-driven mechanisms like tryptophan secretion.

Currently, one focus of our research is on understanding the metabolic interactions among microbial members and their impact on plant phenotype. We highlight that competition among microbial members is essential for establishing root microbiota, preventing fungal dysbiosis, and promoting plant health. We have identified biocontrol strains from the *Pseudomonadaceae* family, particularly two *Pseudomonas* isolates (R401 and R569), which exhibit broad inhibitory activity against fungi and bacteria. Notably, we identified three R401's biosynthetic gene clusters, involved in the production of exometabolites such as DAPG, pyoverdine, and brassicapeptin as important for microbial competition and plant protection. While competitive interactions are common, beneficial collaborations among microbes are less frequent. However, our studies of carbon utilization dynamics in synthetic communities reveal 3-O-methyl-glucose (3OMG) as a crucial resource utilized by *Pseudomonas* R329, with its by-products promoting *Plectosphaerella* growth. Beyond the importance of 3OMG in promoting bacterial-fungal homeostasis, we have identified its role in enhancing plant phenotype by reprogramming pathogenic fungi to a neutral state through the downregulation of carbohydrate-active enzymes.

In conclusion, our findings elucidate the intricate interplay of negative and positive interactions within plant-associated microbial communities, highlighting dependencies on exometabolites that carry significant ecological implications for plant health. This comprehensive understanding of microbial dynamics provides valuable insights for advancing plant-microbe research and enhancing agricultural practices.

Key words : Microbe-microbe interactions, synthetic community, multi-kingdom, exometabolites, carbon reallocation, root microbiota.

Diversity of Molecular Factors of Resistance to Bacterial Wilt (Ralstonia solanacearum) in Eggplant.

Terriane VANHOVE (1), Edith GAROT (1), Stéphane POUSSIER (1), Yann PECRIX (2), Cyril JOURDA (2),

(1) UMR PVBMT, Université de La Réunion, Saint-Pierre, France.

(2) UMR PVBMT, CIRAD, Saint-Pierre, France.

The Ralstonia solanacearum species complex (RSSC) causes the bacterial wilt (BW) on a wide range of hosts including Solanaceae crops such as eggplant. BW is a major disease in tropical and subtropical areas. Varietal resistance is considered the most effective way to sustainably control the BW at a reasonable cost. We studied a double haploid (DH) population of 207 lines, obtained from F1 hybrids [MM1800 x MM738]. The parent S.melongena MM1800 is a resistant S. melongena line created by the AVRDC (Asian Vegetable Research Development Center, Taiwan). MM1800 is resistant to a large range of RSSC strains in particular to phylotype I and IIB-1 strains (Lebeau et al., 2011) . The parent MM738 (susceptible), is a French S. melongena line created by INRAe (Institut National de Recherche pour l'Agriculture, l'alimentation et l'Environnement, France).The DH population and controls were infected with the most prevalent strains of RSSC, phylotype I-31 in summer and II-B1 in winter, using the irrigation inoculation method. Genetic data from an DH population were analyzed using Stacks and a high-density map was developed with 2,846 genotyping-by-sequencing (GBS)-generated single nucleotide polymorphism (SNP) markers using 187 DH progenies from F1 hybrids [MM1800 x MM738]. This analysis will allow us to identify QTLs associated with resistance. This study should provide a useful resource for marker-assisted selection already in place for two Reunion Island eggplant varieties.

References:

Lebeau, A., Daunay, M.-C., Frary, A., Palloix, A., Wang, J.-F., Dintinger, J., Chiroleu, F., Wicker, E., Prior, P., 2011. Bacterial Wilt Resistance in Tomato, Pepper, and Eggplant: Genetic Resources Respond to Diverse Strains in the Ralstonia solanacearum Species Complex. Phytopathology® 101, 154–165. https://doi.org/10.1094/PHYTO-02-10-0048

Phage patrol: an effective targeted biocontrol strategy against Xanthomonas hortorum pv. Vitians

Anaelle BAUD (1), Inès ROUGIS (1), Marie GRAINDORGE (2), Alexandre BURLET (3), Denis COSTECHAREYRE (2), Franck BERTOLLA (1)

(1) Universite Claude Bernard Lyon 1, Laboratoire d'Ecologie Microbienne, UMR CNRS 5557, UMR INRAE 1418, VetAgro Sup, 69622 Villeurbanne, France.

(2) GREENPHAGE SAS, 34380 Clapiers, France.

(3) Centre Technique Interprofessionnel des Fruits et des Légumes, CTIFL, 69126 Brindas, France.

The use of bacteriophages as biocontrol agents of phytopathogenic bacteria at different stages of the agricultural supply chain has shown promising results in recent years (1), notably on Xanthomonas spp (2). Bacterial leaf spot of lettuce caused by Xanthomonas hortorum pv. vitians (Xhv) is a major threat for lettuce producers worldwide due to the lack of effective disease control strategies (3) (4). In order to explore the potential of phages to reduce the severity and incidence of this plant disease, we isolated a collection of twenty-eight phages on a panel of strains representative of the genetic diversity of the pathovar. Based on host range, ease of production, liquid activity, genome analysis, and morphology, six promising phages were selected to formulate the cocktail. The cocktail's inhibitory activity was validated against a broader range of Xanthomonas strains to assess its efficacy and specificity. The phage cocktail inhibited 84% of the Xhv strains tested and had no lytic activity on the phylogenetically closest species. The selected phages' stability was tested under various physico-chemical conditions, including UV radiation, temperature, and pH. These remained stable at 45-70°C and at extreme pH levels, but the quantity decreased drastically after a short exposure to UV light. To improve their stability in field conditions, agricultural adjuvants and UV-blocking agents were tested as phagic cocktail formulations. The efficacy of the phagic cocktail was evaluated in the field with different application frequencies. Importantly, the formulated phage cocktail application did not adversely affect key enzymatic activities associated with the nitrogen cycle, suggesting that phage-based biocontrol can be integrated into agricultural practices without negative impacts on soil health. These results highlight the potential of phages for controlling Xhv, though further optimization is required.

References:

(1) Svircev A, Roach D, Castle A. 2018. Framing the future with bacteriophages in agriculture. Viruses 10:218.

(2) Nakayinga R, Makumi A, Tumuhaise V, Tinzaara W. 2021. Xanthomonas bacteriophages: a review of their biology and biocontrol applications in agriculture. BMC Microbiol 21:291.

(3) Barak D, Koike T, Gilbertson L. 2002. Movement of Xanthomonas campestris pv. vitians in the stems of lettuce and seed contamination', Plant Pathology, 51(4), pp. 506–512.

(4) Koike ST, Gilbertson RL. 2017. Chapter 25: Detection of Xanthomonas campestris pv. vitians in lettuce seeds, p. 173–178. In detection of plant-pathogenic bacteria in seed and other planting material, Second Edition. The American Phytopathological Society.

Water and Immunity – Using a Novel Plant Screen to Identify Candidate Genes

Soline MARTY (1), Caroline BELLENOT (1), Jean-Marc ROUTABOUL (1), Alice BOULANGER (1), Corinne AUDRAN (1), Sébastien CARRERE (1), Marie-Françoise JARDINAUD (1), Patrick LAUFS (2), Lionel NAVARRO (3), Laurent NOËL (1)

(1) University of Toulouse, INRA & CNRS UMR 2594/441, University Paul Sabatier, Laboratory of Plant-Microbe Interactions, 24 chemin de Borderouge-Auzeville, 31320 Castanet-tolosan, France

(2) Institut Jean-Pierre Bourgin (IJPB), Université Paris-Saclay, INRAE & AgroParisTech UMR 1318, Route de St-Cyr (RD10) 78026 Versailles France

(3) Institut de Biologie de l'Ecole Normale Supérieure (IBENS), Centre National de la Recherche Scientifique UMR8197, Institut National de la Santé et de la Recherche Médicale U1024, Paris, France.

Plants set up complex immune responses capable of controlling the proliferation of most microbes which they encounter. For pathogenic microbes, disease symptoms are often associated with the development of water-soaked lesions visible by the accumulation of water in the apoplasm. Though it is well described that humidity is one of the factors favoring the development of plant diseases, the underlying molecular mechanisms are hypothetical. Recent work evidenced the manipulation of water availability in apoplasm by the pathogenic bacterium Pseudomonas syringae (Badel et al., 2007). This raises the question of water being a direct effector of plant immunity itself.

In this study, we identified mutant Arabidopsis plants with increased amounts of apoplastic water and showed increased proliferation of this vascular bacterium Xanthomonas campestris pv campestris (Xcc) in the mesophyll. This raises key questions such as: “Can the plant regulate its water flow to combat bacterial infection?”, “Does Xcc enhance high-humidity conditions to promote its proliferation?” or “What genes are involved in water flow regulation in both the plant and pathogen?” In order to identify plant genes that promote the accumulation of water in the apoplast we are performing a forward genetic screen using a compact fully-sequenced Arabidopsis mutant library, comprising around 900 near-homozygous lines (Capilla-Perez et al., 2018; Carrère et al., 2024). We will present our progress in the search for water-related immunity genes.

References:

Badel JL, Shimizu R, et al. (2007) A Pseudomonas syringae pv. tomato avrE1/hopM1 Mutant Is Severely Reduced in Growth and Lesion Formation in Tomato. MPMI. 19(2) : 99-111.

Capilla-Perez L, Solier V, et al. (2018) The HEM Lines: A New Library of Homozygous Arabidopsis thaliana EMS Mutants and its Potential to Detect Meiotic Phenotypes. Frontiers in Plant Science. 9:1339.

Carrère S, Routaboul JM et al. (2024) A fully sequenced collection of homozygous EMS mutants for forward and reverse genetic 2 screens in Arabidopsis thaliana. The plant Journal.

Fungal leaf communities reduce the development of grapevine downy mildew

Valerie MARTIN (1,2), Aarti JASWA (3), Manon CHARGY (3), Paola FOURNIER (3), Lucile PELLAN (3), Olivier FABREGUETTES (3), Marie-Cécile DUFOUR (3), Jessica VALLANCE (3), François DELMOTTE (3), Corinne VACHER (3)

(1) Utah State University; Logan, Utah, United States

(2) Rocky Mountain Biological Laboratory; Gothic, Colorado, United States

(3) INRAE - UMR1065 Santé et Agroécologie du Vignoble (SAVE)

The wine industry is an essential sector of French agriculture, generating 14% of production by value (1). Unfortunately, viticulture uses a large amount of pesticides, with vineyards receiving an average of 16 treatments per year, 82% of which are intensive fungicide treatments used to control downy mildew (Plasmopara viticola) and powdery mildew (Erysiphe necator) (2). To reduce costs and preserve human and environmental health, reducing pesticide use in grapevine is now a major goal of France. Rapidly achieving zero-pesticide viticulture requires a redesign of cropping systems to enhance disease prevention, including by diversifying biocontrol strategies (3). Current microbial biocontrol methods consist of single microbial strains applied in high concentrations to plant tissues. However, plant-inhabiting microbial communities harbor dozens to hundreds of microbial strains (4). Furthermore, recent studies have shown that microbial communities with higher taxonomic and phylogenetic diversity exhibit increased resistance to invasion by new species, such as a pathogen (5). We set out to test whether growth and asexual reproduction by Plasmopara viticola, the oomycete pathogen that causes grapevine downy mildew (6), is inhibited by taxonomically or phylogenetically diverse synthetic communities of leaf-dwelling bacteria and fungi. We created synthetic microbial communities (SynComs; (7)) differing in taxonomic and phylogenetic diversity, investigated whether these SynComs can colonize foliar disks of grapevine, and assessed the effects of SynComs on downy mildew development using in vitro assays. Our preliminary results suggest that fungi are the most involved in disease reduction and that the phylogenetic diversity of fungal communities, but not that of bacteria, hinders sporangial development. These findings contribute to a growing understanding of pathogen-microbiome interactions and to the development of new biocontrol strategies for grapevine downy mildew.

References:

(1) Delmotte et al. (2019) INRAE Scientific Submission Form for VITAE.

(2) Beaufils et al. (2021). GRAPH'AGRI: L'agriculture, la forêt, la pêche et les industries agroalimentaires.

(3) Jacquet et al. (2022). Agronomy for Sustainable Development.

(4) Barroso-Bergadà et al. (2021). PhytoFrontiers.

(5) Mallon et al. (2015). Trends in microbiology.

(6) Fontaine et al. (2021). Current Biology.

(7) Vorholt et al. (2017). Cell Host & Microbe.

Description of the bacterial and fungal microbiota of rice field soils with antagonistic effects against the rice root-knot nematode Meloidogyne graminicola in Cambodia

Michel BARBIER(1,3) - Kimsrong UON(2) - Cécile PERROLLAZ(1,3) - Jamel ARIBI(1)

Pierre CZERNIC (1,3) - Malyna SUONG(2,3) - Lionel MOULIN(1,3) - Stéphane BELLAFIORE(1,3)

(1) Plant Health Institute, Univ Montpellier, IRD, CIRAD, INRAe, Institut Agro, Montpellier, France

(2) Institute of Technology of Cambodia, Phnom Penh, Cambodia

(3) International Laboratory of Excellence in Co-engineering for Sustainable Agrosystems, ITC, RUA, NUBB, IRD, CIRAD, Phnom Penh, Cambodia

Meloidogyne graminicola is a plant-parasitic nematode widespread in Southeast Asian rice fields, where it can cause yield losses of around 80% of production, making it a threat to rice cultivation. To control Meloidogyne spp., synthetic nematicidal molecules such as methyl bromide have long been used, but their use and commercialisation is increasingly restricted due to their harmful effects on health and the environment. It's therefore necessary to develop new alternative methods of controlling plant-parasitic nematodes for sustainable agriculture.

In Cambodia, there are rice fields under conservation agriculture where rice yields are better than neighbouring fields and where rice has significantly lower M. graminicola infestation despite its presence in the soil. These soils are probably suppressive, meaning that they are naturally able to reduce the impact of the disease in the field. The question is whether this specific activity is carried by microorganisms or not, and if so, which microorganisms are responsible. Studying these soils could lead to the discovery of new biological control agents.

During my PhD, we sampled soils in these suppressive rice fields and in two other Cambodian provinces and then developed a method to detect in vitro antagonistic effects against M. graminicola. We characterised the soil bacterial and fungal microbiota using a metabarcoding approach to identify microorganisms correlated with soil suppressive activity. Two sampled sites showed antagonistic activities, in which we identified the nematode-trapping fungi genus Arthrobotrys sp. and the hyperparasitic bacteria Pasteuria sp. known to control plant-parasitic nematodes. These activities were also correlated with an enrichment of the genus Lysinibacillus, described as nematicidal against Meloidogyne spp.. A culturomic approach should allow us to isolate these microorganisms to assess their biocontrol capacities in planta, with a view to using them in fields in the future.

Towards a more sustainable agriculture, Sphingomonas sediminicola a potential bio-inputs to reduce chemicals inputs in a local crop rotation

Audrey PECOURT (1,2) , Audrey BERTRAND (1,2), Kamar HAMADE (3), Manuella CATTEROU (1,2) , François MESNARD (2,4), Vivien SARAZIN (2,3), Frédéric DUBOIS (1,2), Jérôme DUCLERCQ (1,2)

(1) Unité EDYSAN - UMR CNRS 7058, Université de Picardie Jules Verne, Amiens, France

(2) Equipe mixtes laboratoire-entreprise RESISTE, Université de Picardie Jules Verne, Amiens, France

(3) AgroStation, Aspach-le-Bas, France

(4) UMRT INRAE 1158 BioEcoAgro, Laboratoire BIOPI, University of Picardie Jules Verne, Amiens, France

Current agricultural practices rely heavily on chemical inputs such as synthetic fertilizers, pesticides, and fungicides. While the overuse of mineral fertilizers has boosted yields, it has also disrupted ecological balance and degraded soils (Barros-Rodríguez et al., 2021). A promising alternative is the use of bacterial bio-inputs, which can reduce dependence on mineral inputs without compromising crop performance.

In the INITIATED project, the bacterial bio-input Sphingomonas sediminicola was tested in a wheat-flax-potato rotation system with cover crops between the main crops. This presentation will focus on the wheat cycle, where S. sediminicola was applied with or without reduced fertilization. The primary objective was to assess whether this bacterial bio-input could maintain wheat yields despite reduced fertilization, influence plant physiology, and affect soil microbial biodiversity. Plant development and physiology were monitored through phenotypic measurements, chlorophyll content analysis, and untargeted metabolomics, with yield parameters evaluated at harvest.

Additionally, the project tracks the long-term persistence of S. sediminicola in the soil using qPCR and analyzes shifts in soil microbial communities through metabarcoding. This presentation will explore the results from the wheat cycle, highlighting the potential of bacterial bio-inputs in sustainable farming.

References:

Barros-Rodríguez, A., Rangseekaew, P., Lasudee, K., Pathom-aree, W., & Manzanera, M. (2021). Impacts of Agriculture on the Environment and Soil Microbial Biodiversity. Plants, 10(11), 2325. https://doi.org/10.3390/plants10112325

Developing biocontrol products against broomrapes: from lab to field.

LURTHY Tristan (1), WOLFF Léa (1), PEROT Ségolène (1), WISNIEWSKI-DYÉ Florence (1), COMTE Gilles (1), REY Marjolaine (1), MERCIER Pierre-Edouard (1), TAIBI Ahmed (2), DREUX-ZIGHA Assia (2), PRIGENT-COMBARET Claire (1).

(1) UMR CNR 5557 Ecologie Microbienne - University Claude Bernard Lyon 1, Villeurbanne, France

(2) Greencell SA - Groupe Greentech - Biopôle Clermont-Limagne, Saint-Beauzire, France

Broomrapes are obligate root holoparasitic plants belonging to Orobanche and Phelipanche genera in the Orobanchaceae family. They cause significant damage in Europe, particularly in Eastern Europe and in the Mediterranean region, leading to important yield and economical losses on a wide range of crops worldwide (rapeseed, sunflower, tobacco, tomato, etc). There is currently no effective way to control this weed. Crop protection strategies against parasitic weeds are mostly based on the use of non-selective chemical pesticides in combination with peculiar plant genotypes 'with good behavior' (tolerant) or of non-host plants in rotation to induce Orobanche seed suicidal germination. In soil, the rhizosphere community plants harbours bacteria that may be able to interfere with the molecular dialogue established between the parasite and its host prior to the penetration of the parasite inside host tissues. We thus explored, in the WeedsBiocontrol project (ANR ECOM-2019-0002), the use of biocontrol rhizobacteria of the Pseudomonas genus as a biological weapon to limit broomrape infestation. We identified candidate strains with Orobanche Germination Inhibition (OGI) activity. By combining comparative genomics and metabolomic profiling, several OGI metabolites were identified. Biocontrol strains with the greatest OGI activity were then produced first in lab-scale fermenters, followed by scaling up to a 100L production pilot. Various growth conditions were tested to optimize cell biomass. Subsequently, greenhouse assays evaluated the protective effects on rapeseed against P. ramosa and on sunflower against O. cumana, allowing the selection of the most effective strain for optimized pilot production. Practical application methods (product quantity, number of applications, optimal plant stage) were defined. Then, field experiments were conducted in Western (rapeseed) and Southwest France (sunflower), for assessing the efficacy of the product in naturally contaminated fields

References:

Lurthy, T, Perot S, Gerin-Eveillard F, Rey M, Wisniewski-Dyé F, Vacheron J, Prigent-Combaret C. 2023. Inhibition of broomrape germination by 2,4-diacetylphloroglucinol produced by environmental Pseudomonas. Microb Biotechnol. 16(12):2313-2325. doi: 10.1111/1751-7915.14336.

POSTERS

Tuesday 14th: poster session for odd numbers/ numéros impairs

Wednesday 15th: poster session for even numbers / numéros pairs

Nom / Name	Poster number
DARRASSE	**1**
CHARGY	**2**
DUFOUR	**3**
ROBENE	**4**
THONON	**5**
BOUTIGNY	**6**
CABRE	**7**
DEGRAVE	**8**
DOLLY	**9**
PECOURT	**10**
SIBY	**11**
COCIANCICH	**12**
GUEGUEN	**13**
HILLAIRET	**14**
DAGVA	**15**
ILYAS	**16**
BOUTRON	**17**
DAVIDDI	**18**
DESBROSSES	**19**
DORE	**20**
HALTER	**21**
RIGOLET	**22**
CELLIER	**23**
BARNY	**24**
BROTTIER	**25**
CIGNA	**26**
DITTMER *Arsenophonus*	**27**
MENDEZ BRAVO	**29**
ZARATE-CHAVES	**30**
BOUBAKRI	**31**
CHADELAUD	**32**
DENDENE	**33**
KOEBNIK DokuWiki	**34**
ZANNIS-PEYROT	**35**
CESBRON	**36**
CUNTY	**37**
DITTMER *Xf multiplex*	**38**
FISCHER-LE SAUX	**39**
ROUTABOUL	**40**
DROGUE	**41**
KOEBNIK *Xylophilus*	**43**
DIAB	**44**
PRUDOT D'AVIGNY	**45**
BARRET *Pantoea*	**46**
BARRET seedling	**47**
LE SIOU	**48**
MIRGUET	**49**
PRIGENT-COMBARET	**50**
ROUX	**51**
TAILLEFER	**52**
PORTIER	**53**
NOEL (Alba)	**54**
VANDELLE	**55**
FOUCHER	**56**
RUH	**57**
BERGE	**58**
SARNIGUET	**59**

POSTER P 1

Seed transmission of Pseudomonas syringae strains responsible for zucchini vein clearing disease

Caroline LACAULT, Stacy ROUSSE, Marie-Agnès JACQUES, and Armelle DARRASSE

Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, F-49000 Angers, France.

Zucchini vein clearing (VCZ) disease affects plantlets grown in nurseries and includes symptoms such as necrosis, vein clearing, growth delays and stunting, but no epidemics has been reported in France on adult plants. This seed-borne disease is caused by bacteria belonging to different genetic lineages (clusters A to E) of the Pseudomonas syringae species complex (Pssc). Cluster-A strains have narrow and others (B-E) wide host ranges within Cucurbitaceae, in line with their type III effector repertoires. Frequency of contaminated seed lots has been increasing in the last 20 years and knowledge about VCZ epidemiology remains limited. The aim of this study was to understand how P. syringae strains contaminate seeds. Different seed transmission routes were tested for two strains having contrasting host ranges (clusters A and E). Floral and vascular routes were used by both strains, but only the cluster E strain was able to transmit by contact. An epidemiological survey in zucchini seed multiplication plots was performed in association with testing initial and harvested seed lots. No bacteria from cluster A to E were recovered in the initial lots by qPCR detection tests after an enrichment phase on culture medium. However, DNA from strains with a wide host range was detected in some initial lots, and bacteria of the same type were found in the corresponding harvested lots. However, surveys of the plots revealed the presence of other bacteria belonging to the Pssc, but no VCZ strains. The origin of cluster-A strains, predominant in seed lots, could not be determined. These results are discussed in relation to seed production areas, control methods and thresholds of detection tools.

References:

Monitoring the establishment of a synthetic microbial community with a potential biocontrol activity against grapevine downy mildew using a microfluidic qPCR chip

Manon CHARGY (1), Marie-Cécile DUFOUR (1), Aarti JASWA (1), Valerie MARTIN (2), Jessica VALLANCE (1), Simon LABARTHE (3), Corinne VACHER (1)

1) UMR SAVE (Santé et agroécologie du vignoble), INRAE, Villenave d'Ornon

(2) Utah State University, Logan

(3) UMR BIOGECO (Biodiversité,gènes et communautés), INRAE, Cestas

Grapevine downy mildew, caused by the oomycete Plasmopara viticola, is responsible for significant economic losses each year, which can lead to yield losses of up to 90% (Toffolatti et al., 2018). In order to limit the use of pesticides that are incompatible with the development of sustainable viticulture, but still very widely used, biocontrol solutions based on the creation of synthetic communities of microorganisms (SynComs) are gradually emerging (Marín et al ., 2021).

In the present study, we designed a synthetic microbial community using a collection of microorganisms isolated from grapevine leaves by a culturomic approach. This synthetic community is made of 43 microbial taxa (including bacteria, yeasts and filamentous fungi) that are either known to have a biocontrol role against plant pathogens, or known to be abundant on grapevine leaves.

In order to monitor the establishment of this community on grapevine leaves, a quantitative PCR (Polymerase Chain Reaction) microfluidic chip was developed. Primers specific to each of the 43 microbial taxa have been designed in single copy housekeeping genes. Microfluidics offers the advantage of carrying out a large number of reactions quickly and at lower cost compared to a classic quantitative PCR system (Kleyer et al., 2017).

In this talk, I will present the first results obtained using the microfluidic chip, opportunities of improvement and perspectives of application to the field. We envision using this tool to monitor vineyard health, as some of the microorganisms targeted by the chip have been found to be related with low susceptibility to downy mildew(Fournier et al., submitted).

References:

Kleyer H, Tecon R, Or D (2017) Resolving Species Level Changes in a Representative Soil Bacterial Community Using Microfluidic Quantitative PCR. Front Microbiol 8: 2017

Marín O, González B, Poupin MJ (2021) From Microbial Dynamics to Functionality in the Rhizosphere: A Systematic Review of the Opportunities With Synthetic Microbial Communities. Front Plant Sci. doi: 10.3389/fpls.2021.650609

Toffolatti SL, Russo G, Campia P, Bianco PA, Borsa P, Coatti M, Torriani SF, Sierotzki H (2018) A time-course investigation of resistance to the carboxylic acid amide mandipropamid in field populations of Plasmopara viticola treated with anti-resistance strategies. Pest Manag Sci 74: 2822–2834

Fournier P., Pellan L., Jaswa A., Cambon M., Chataigner A., Bonnard O., Raynal M., Debord C., Poeydebat C., Labarthe S., Delmotte F., This P., Vacher C. Revealing microbial consortia that interfere with grapevine downy mildew through microbiome epidemiology. Submitted

Effect of the biocontrol agent Pythium oligandrum on grapevine trunk diseases and rhizosphere microbial communities

Séverine Lopez, Alexandre Chataigner, Jessica Vallance and Marie-Cécile Dufour

INRAE, Bordeaux Sciences Agro, ISVV, SAVE, 33140 Villenave d'Ornon, France

Grapevine trunk diseases (GTDs) are a major concern in viticulture. Since the ban of sodium arsenate in 2001, the development of alternative biocontrol methods has become a major challenge. The oomycete Pythium oligandrum is among the most promising biocontrol agents, as it is known to improve vine health by strengthening natural defenses and can reduce diseases by up to 40% (Gerbore et al, 2014; Yacoub et al, 2016 and 2020). In the present study, we evaluated the efficiency of a biosolution formulated from P. oligandrum on GTDs. We evaluated efficiency at different scales, from the nursery to vineyards, and we assessed the environmental impact of the biosolution to ensure its safety. As part of this environmental impact assessment, we performed a three-month experiment in semi-controlled conditions to assess the impact of the biosolution on rhizosphere bacterial and fungal communities using a high-throughput sequencing approach. Vines were treated with the biosolution and were inoculated with two fungi involved in GTDs: Neofusicoccum parvum (involved in Botryosphaeria Dieback) and Phaeomoniella chlamydospora (involved in Esca). Our results confirmed that P. oligandrum has little impact on the composition of rhizosphere microbial communities (Vallance et al, 2012). However, the treatment increased the abundance of some specific taxa, that are known to act as plant growth promoting rhizobacteria (PGPR) or biocontrol agents. This could explain the decrease in necroses triggered by the two pathogens N. parvum and P. chlamydospora after application of the biosolution.

References:

Gerbore J., Benhamou N., Vallance J., Le Floch G., Grizard D., … Rey P., 2014. Biological control of plant pathogens: advantages and limitations seen through the case study of Pythium oligandrum. Environmental Science and Pollution Research 21: 4847–4860. DOI: 10.1007/s11356-013-1807-6.

Vallance J., Déniel F., Barbier G., Guerin-Dubrana L., Benhamou N., Rey P., 2012. Influence of Pythium oligandrum on the bacterial communities that colonize the nutrient solutions and the rhizosphere of tomato plants. Canadian Journal of Microbiology 58: 1124–1134. DOI: 10.1139/w2012-092.

Yacoub A., Gerbore J., Magnin N., Chambon P., Dufour M.-C., … Rey P., 2016. Ability of Pythium oligandrum strains to protect Vitis vinifera L., by inducing plant resistance against Phaeomoniella chlamydospora, a pathogen involved in Esca, a grapevine trunk disease. Biological Control 92: 7–16. DOI: 10.1016/j.biocontrol.2015.08.005.

Yacoub A., Haidar R., Gerbore J., Masson C., Dufour M.-C., … Rey P., 2020. Pythium oligandrum induces grapevine defence mechanisms against the trunk pathogen Neofusicoccum parvum. Phytopathologia Mediterranea 59: 565–580. DOI: 10.14601/Phyto-11270.

POSTER P 4

Development of real-time LAMP assays for in-field detection of three bacterial vascular banana diseases

Isabelle ROBENE (1,2), V. MAILLOT-LEBON (1), Samuel ROZSASI (1,2), Diane MOSTERT (2), Altus VILJOEN (2), Yolande CHILIN-CHARLES (3), Sheryl BOTHMA (2), Camilo GIANINAZZI (4),Emmanuel WICKER (5), Mikel ARRIETA-SALGADO (5)

1CIRAD, UMR PVBMT, F-97410 Saint-Pierre, Réunion Island, France.

2Department of Plant Pathology, Stellenbosch University, Stellenbosch 7602, South Africa.

3CIRAD, UMR PHIM, F-97130 Capesterre-Belle-Eau, Guadeloupe, France.

4 Qualiplante SAS, Cap Alpha,34830 Clapiers, France.

5CIRAD, UMR PHIM, F-34398 Montpellier, France.

Bacterial diseases of banana are becoming increasingly significant globally, resulting in reduced yields and higher disease management costs. The most important bacterial diseases include Moko and banana blood disease (BDB), caused by Ralstonia solanacearum and R. syzygii subsp. celebesensis, respectively, and banana Xanthomonas wilt (BXW), caused by Xanthomonas vasicola pv. musacearum. Effective surveillance and disease management require available Point-of-Care (POC) diagnostics for on-site operation. Pathogen-specific DNA targets were designed using in silico comparative genomic analysis of target and non-target genomes. Simplex real-time LAMP (Loop-mediated isothermal amplification) assays were successfully developed for Xanthomonas vasicola pv. musacearum and R. syzygii subsp. celebesensis. Due to the high genetic diversity within the bacterium causing Moko disease, a duplex LAMP assay targeting two DNA regions was developed to detect all sequevars of the pathogen. Each LAMP assay demonstrated 100% specificity when tested against a wide range of target and non-target strains, including closely related taxa. High sensitivity was demonstrated for all LAMP assays when assayed on spiked banana tissue, with a limit of detection (100% positive) of 10^4 CFU/ml (50 target copies per reaction). Each LAMP assay successfully detected the target bacteria from banana plants inoculated under controlled conditions. A simplified DNA extraction method and the different LAMP assays were also validated in the field, demonstrating the portability of this technology. Moreover, highly reliable results were achieved during inter-laboratory testing. All-inclusive commercial kits are currently manufactured. The availability of rapid POC diagnostic tools will enable quick identification of banana wilt diseases in the field, improving disease management strategies.

References:

POSTER P 5

Genotype specific responses of Musa spp. baseline phytomicrobiome to Fusarium oxysporum f. sp. cubense infection

Salomé THONON (1), Claude BRAGARD (1), Anne LEGREVE (1)

(1) UCLouvain/Earth and Life Institute/ELIM/Plant Health Laboratory, Louvain-la-Neuve, 1348, Belgium

Banana (Musa spp.), a major crop providing food security to millions of people, is highly threatened by many pests and pathogens. A particularly devastating soil-borne pathogen is Fusarium oxysporum f. sp. cubense (Foc), responsible of the banana Fusarium wilt (Panama disease).

Against this destructive pathogen, the importance of the phytomicrobiome in supporting plant health is increasingly acknowledged. Banana associated microorganisms, and in particular endophytes, have become the focus for developing disease protection strategies. Recently, the presence of a core microbiome closely associated with Musa spp., where keystone species could play major roles for pathogen control, was suggested.

Control strategies based on such keystone species are highly promising. Yet, many challenges remain to fully decipher the functioning of banana endophytic microbiome. How do endophytes mitigate disease ? Can they provide a sustainable and effective control against Foc ?

To address these questions, two local banana cultivars in Benin, sensitive and resistance to Foc race 1 respectively, are studied. Samples were collected from several infested sites for DNA extraction and endophytes isolation. Taking advantage of new sequencing technologies, bacterial and fungal core endophytic microbiomes are being investigated. The dysbiosis resulting from Foc infection is being evaluated, to identify of keystone species that could be related to resistance against the pathogen.

This will open doors to improve understanding on the underlying interactions involved in pathogen suppression. The role of these keystone species will be further investigated, first with a culture-dependent approach using confocal microscopy. A complementary and innovative culture-independent approach, involving plant grafting and metagenomics, will also be explored.

This research will pave the way towards the development of effective phytomicrobiome-based control.

References:

(1) UCLouvain/Earth and Life Institute/ELIM/Plant Health Laboratory, Louvain-la-Neuve, 1348, Belgium

Test performance study for the detection of Xylella fastidiosa and the identification of subspecies in dormant plant species

Anne-Laure BOUTIGNY, Ugo MOUISSE, Bruno LEGENDRE, Valérie OLIVIER

Anses, Plant Health Laboratory, BVO unit, Angers, France

As part of the collaborative European phytosanitary research network “Euphresco”, a test performance study (TPS) was conducted in 2023 to evaluate the performance of several molecular methods for the detection of Xylella fastidiosa and the identification of subspecies in naturally infected dormant plant samples.

The TPS was organized by Anses and a total of 14 laboratories from 10 countries participated. The panels sent to participants contained samples of dormant woody twigs naturally infected with different subspecies and sequence types of X. fastidiosa and healthy twigs. Twigs come from almond and plum trees, and vines, and were collected in France, Italy, Spain or Israel. A common protocol for Xf detection (CTAB DNA extraction / Harper PCR) was proposed by the organizer and it was possible for participants to test other DNA extraction and PCR protocols to detect X. fastidiosa and identify the subspecies. In addition, some laboratories tested MLST to determine the sequence types and digital PCR to detect and quantify X. fastidiosa.

After verifying the homogeneity and stability of the samples, the performance criteria (diagnostic sensitivity, diagnostic specificity, accuracy, repeatability and reproducibility) for each protocol were calculated. The “Harper simplex” PCR method was validated for the detection of X. fastidiosa and the “Hodgetts simplex” and the “Dupas teraplex” PCR methods were validated for the identification of subspecies in dormant plant samples. Other methods are usable to detect Xf and identify the subspecies reliably in dormant plants but they were tested by only 1 or 2 laboratories. The results of this collaborative work lead to common standards for the detection of X. fastidiosa in dormant plants; which will be proposed for the next versions of the EPPO PM7/24 and ICPP DP25 diagnostic protocols for X. fastidiosa.

References:

PM 7/24 (5) (2023), Xylella fastidiosa. EPPO Bulletin, 53: 205–276. https://doi.org/10.1111/epp.12923.

Investigation of PTI activation following the perception of an elicitor combination, in Arabidopsis thaliana

L. CABRE (1), L. JING (2), M. MAKECHEMU (3), K. HELUIN (4), S. ELKHAMLICHI (4), J. LEPRINCE (5), MC KIEFER-MEYER (4), S. PLUCHON (1), C. ZIPFEL (3), JC MOLLET (4), E. NGUEMA-ONA (1)

1 Agro Innovation International TIMACAGRO, Laboratoire de Nutrition Végétale. Saint Malo, France

2 Agro Innovation International TIMACAGRO, Plateforme Analytique de recherche, Saint Malo, France

3 Department of Plant and Microbial Biology, University of Zürich, Zollikerstrasse 107CH-8008 Zürich

4 Univ Rouen Normandie, GLYCOMEV UR 4358, SFR Normandie Végétal FED 4277, Innovation Chimie Carnot, IRIB, F-76000 Rouen, France

5 Univ Rouen Normandie, CNRS, INSERM, HERACLES US 51 UAR 2026, PRIMACEN, IRIB, F-76000 Rouen, France

Plant elicitors (PE) have the ability to activate pattern triggered immunity (PTI), via there recognition by pattern recognition receptors (PRRs). Early transcriptional responses to distinct PAMPs are mostly overlapping, regardless of the elicitor being used. However, it remains poorly known if the same patterns are observed for metabolites and proteins produced later during PTI. In addition, little is known about the impact of a combination of elicitors on PTI and the level of induced resistance to pathogens.

Here, we evaluated how the combination of a bacterial and a fungal elicitor, perceived by different PRRs, affect A. thaliana PTI activation. We monitored A. thaliana resistance to the bacterial pathogen Pseudomonas syringae pv. tomato DC3000 following application of flg22 and chitosan elicitors, used individually or in combination. We also investigated the effect of these treatments on the metabolome by using an untargeted analysis. We found that elicitors combination impacts a highest number of metabolites and deregulates specific metabolites pathway, when compared to the elicitors used alone. This highlights the interest to use a combination of elicitors in crop protection strategies.

References:

Cabre, L., Jing, L., Makechemu, M., Heluin, K., El Khamlichi, S., Leprince, J., et al. (2024) Additive and Specific Effects of Elicitor Treatments on the Metabolic Profile of Arabidopsis thaliana. MPMI, 37, 112–126

Apple defense lectins are involved in resistance to Erwinia amylovora, functional validations approaches

BODELOT Antoine (1), DOUSSET Nicolas (1), RAVON Elisa (1), HEINTZ Christelle (1), DEGRAVE Alexandre (1), VERGNE Emilie (1)

(1) Institut Agro, Univ Angers, INRAE, IRHS, SFR QUASAV, F-49000 Angers, France

Plant defense protein-encoding genes, such as Pathenogenesis-Related (PR) genes are extensively used as molecular markers to dissect the signaling cascades leading to plant defense responses. However, studies focusing on the biochemical or biological properties of the encoded proteins remain rare. We identified a class of apple (Malus domestica) genes, named M. domestica AGGLUTININS (MdAGGs), that is highly expressed upon defense elicitation by the plant resistance inducer acibenzolar S-methyl (Chavonet et al., 2022). These 17 highly-conserved MdAGG genes encode proteins of the amaranthin-like lectin family, glycan-binding proteins that are widely distributed in plants but whose function remain unknown. Previous studies showed that Erwinia amylovora (Ea), the fire blight pathogen, actively represses MdAGGs gene expression thanks to its type 3 secretion system. Moreover, recombinant MdAGG proteins were shown to agglutinate bacterial cells in vitro, and that the bacterial exopolysaccharide amylovoran could prevent this aggregation, leading to the hypothesis that MdAGGs are key component of apple's resistance response toward Ea. Functional validation by loss of function using CRISPR-Cas9 edition combined to gain of function approaches using cisgenesis were undertaken in apple to determine if these sticky proteins could modulate the outcome of the fire blight disease.

References:

Chavonet E., Gaucher M., Warneys R., Bodelot A., Heintz C., Juillard A., Cournol R., Widamalm G., Bowen J.K., Hamiaux C., Brisset M.N., Degrave A. (2022) Search for Host Defense Markers Uncovers an Apple Agglutination Factor Corresponding with Fire Blight Resistance. Plant Physiology 188, 2: 1350–68. https://doi.org/10.1093/plphys/kiab542

Toward a formulation of a new biocontrol agent against blackleg and soft-rot diseases for potato fields

Dolly Maurane (1), Jérémy Cigna (2), Yannick Raoul des Essarts (1), Denis Faure (1)

(1) Institut for Integrative Biology of the Cell (I2BC), Paris-Saclay University, CEA, CNRS

(2) French Federation of Seed Potato Growers (FN3PT/inov3PT)

The Pectobacterium and Dickeya bacterial species cause blackleg and soft-rot diseases on potato plants and tubers. Prophylactic approaches like biocontrol are important to conserve a high quality of seed potato tubers but difficult to implement in the face of pathogen diversity [1].

In this study, we present the identification and characterization of new biocontrol agents. Successive screenings of 10,000 bacterial isolates resulted in the selection of 17 strains with growth-inhibiting properties against the 4 tested Dickeya and Pectobacterium species. In vitro assays revealed a fitness decrease of these pathogens in the presence of 6 strains namely Pseudomonas fluorescens PA4C2, Pseudomonas fluorescens PA3G8, Pseudomonas sp. PA14H7, Bacillus simplex BA2H3 and Pseudomonas brassicacearum PA1G7 and PP1-210F.

To represent the pathogen species diversity, we evaluated the efficiency of these 6 biocontrol agents against a wider range of recently collected pathogens. A total of 41 isolates from 11 pathogen species, including 4 classically isolated in fields, were tested. Among the biocontrol agents tested, the Pseudomonas sp. PA14H7 strain was the most active [2].

In view of these results, formulating the biocontrol agent to be directly applied in fields could be interesting. In vitro, we evaluated the viability of Pseudomonas sp. PA14H7 formulation and its biological activity against pathogens. In addition, a strain-specific qPCR molecular tool was designed to be able to detect and quantify the biocontrol agent in greenhouse and field assays over time. The persistence of Pseudomonas sp. PA14H7 in an inoculated soil was analyzed.

Eventually, the Pseudomonas-formulated biocontrol agent PA14H7 could be used as a strategy to limit soft-rot and blackleg diseases caused by the Pectobacterium and Dickeya on potato fields.

References:

[1] Cigna, J., Robic, K., Dewaegeneire, P., Hélias, V., Beury, A., & Faure, D. (2023). Efficacy of Soft-Rot Disease Biocontrol Agents in the Inhibition of Production Field Pathogen Isolates. Microorganisms, 11(2), 372.

[2] Raoul des Essarts, Y., Cigna, J., Quêtu-Laurent, A., Caron, A., Munier, E., Beury-Cirou, A., ... & Faure, D. (2016). Biocontrol of the potato blackleg and soft rot diseases caused by Dickeya dianthicola. Applied and environmental microbiology, 82(1), 268-278.

Exploring the potential of Sphingomonas bio-inputs for wheat drought resilience under climate change

Audrey PECOURT (1,2), Audrey BERTRAND (1,2), Gaëlle MONGELARD (3) , Valentin AVIT (4) Kamar HAMADE (4), Herve DEMAILLY (3), Manuella CATTEROU (1,2), Laurent GUTIERREZ (3), François MESNARD (2,5), Vivien SARAZIN (2,4), Frédéric DUBOIS (1,2), Jérôme DUCLERCQ (1,2)

1Unité EDYSAN - UMR CNRS 7058, Université de Picardie Jules Verne, Amiens, France

2Equipe mixtes laboratoire-entreprise RESITE, Université de Picardie Jules Verne, Amiens, France

3Centre de Ressources Régionales en Biologie Moléculaire (CRRBM), Université de Picardie Jules Verne, Amiens, France

4AgroStation, Aspach-le-Bas, France

5UMRT INRAE 1158 BioEcoAgro, Laboratoire BIOPI, Université de Picardie Jules Verne, Amiens, France

Rising temperatures, shifting rainfall patterns, and increasing extreme weather events are projected to significantly impact wheat production by the middle and end of the century (Challinor et al., 2014; Zhao et al., 2017). These climate change drivers can directly or indirectly influence soil microbial communities. Bacterial bio-inputs, however, represent a promising adaptation strategy for mitigating the effects of global warming on plants (De Vries & Griffiths, 2018; Jansson & Hofmockel, 2020).

Our study explores the potential of two bacterial bio-inputs, Sphingomonas sediminicola and Sphingomonas daechungensis, applied to wheat under water deficit conditions. The primary objective was to evaluate their capacity to promote wheat growth and modulate plant physiology. The second goal was to assess their potential to protect wheat from drought-induced stress. Plant development and physiology were monitored using a high-throughput phenotyping platform (PlantScreenTM), complemented by measurements of stress metabolite levels and transcriptomic analyses. This research underscores the promising role of bacterial bio-inputs in enhancing wheat's resilience to climate change, offering insights into sustainable agricultural practices for future climatic challenges.

References:

•Challinor, A. J., Watson, J., Lobell, D. B., Howden, S. M., Smith, D. R., & Chhetri, N. (2014). A meta-analysis of crop yield under climate change and adaptation. Nature Climate Change, 4(4), 287-291. https://doi.org/10.1038/nclimate2153

•De Vries, F. T., & Griffiths, R. I. (2018). Impacts of Climate Change on Soil Microbial Communities and Their Functioning. In Developments in Soil Science (Vol. 35, p. 111-129). Elsevier. https://doi.org/10.1016/B978-0-444-63865-6.00005-3

•Jansson, J. K., & Hofmockel, K. S. (2020). Soil microbiomes and climate change. Nature Reviews Microbiology, 18(1), 35-46. https://doi.org/10.1038/s41579-019-0265-7

•Zhao, C., Liu, B., Piao, S., Wang, X., Lobell, D. B., Huang, Y., Huang, M., Yao, Y., Bassu, S., Ciais, P., Durand, J.-L., Elliott, J., Ewert, F., Janssens, I. A., Li, T., Lin, E., Liu, Q., Martre, P., Müller, C., … Asseng, S. (2017). Temperature increase reduces global yields of major crops in four independent estimates. Proceedings of the National Academy of Sciences, 114(35), 9326-9331. https://doi.org/10.1073/pnas.1701762114

From isolation to innovation : Xanthomonas hortorum disease on lettuce and its associated phages

Heimrick SIBY (1) ; Isak ALTUN ; Laurette HAUROGNE ; Eric NGUEMA-ONA ; Sylvain PLUCHON

(1) Agro Innovation International

Bacterial leaf spot of lettuce, caused by Xanthomonas hortorum pv. vitians, represents a significant threat to agriculture economy leading to substantial economic losses (Sahin et al., 2002 ; Ozyilmaz et al., 2018). The lack of effective treatments to eliminate the bacterium has led to the search for new sustainable control strategies (Silva et al., 2021). Bacteriophages, viruses that specifically infect and kill bacteria, present a potential biocontrol strategy against this pathogen (Buttimer et al., 2017). The aim of the project was to develop pathotest to study the Xanthomonas hortorum disease. Disease symptoms were reproduced in a greenhouse using Lactuca sativa KIRINIA, inoculated with Xanthomonas hortorum pv. vitians from the collection of INRAE Pays de la Loire UMR IRHS. Necrotic brown spots were observed in the inoculated group. Following the observation of disease symptoms in a controlled environment, the next step was to investigate wild strains of the pathogen. Samples were collected from agricultural fields in Normandy, leading to the isolation of several bacterial strains. Two strains of Xanthomonas were characterized through genomic analysis. Simultaneously, bacteriophages were isolated from plant samples, and their bacteriolytic activity was tested against the wild-type Xanthomonas strains. These preliminary results demonstrate the potential of this test for screening the effectiveness of phage solutions against the disease.

References:

Buttimer, Colin, Olivia McAuliffe, R. P. Ross, Colin Hill, Jim O'Mahony, et Aidan Coffey. « Bacteriophages and Bacterial Plant Diseases ». Frontiers in Microbiology 8 (20 janvier 2017). https://doi.org/10.3389/fmicb.2017.00034.

Ozyilmaz, Umit, et Kemal Benlioglu. « Bacterial Leaf Spot of Lettuce Caused by Xanthomonas Hortorum Pv. Vitians in the Aegean Region of Turkey ». Australasian Plant Disease Notes 13, no 1 (décembre 2018): 37. https://doi.org/10.1007/s13314-018-0325-2.

Sahin, F., P. A. Abbasi, M. L. Lewis Ivey, J. Zhang, et S. A. Miller. « Diversity Among Strains of Xanthomonas Campestris Pv. Vitians from Lettuce ». Phytopathology® 93, no 1 (janvier 2003): 64 70. https://doi.org/10.1094/PHYTO.2003.93.1.64.

Silva, Aminthia Pombo Sudré, Fábio Lopes Olivares, Cláudia Pombo Sudré, Lázaro Eustáquio Pereira Peres, Natália Aguiar Canellas, Rakiely Martins Da Silva, Vicenza Cozzolino, et Luciano Pasqualoto Canellas. « Attenuations of Bacterial Spot Disease Xanthomonas Euvesicatoria on Tomato Plants Treated with Biostimulants ». Chemical and Biological Technologies in Agriculture 8, no 1 (décembre 2021): 42. https://doi.org/10.1186/s40538-021-00240-9.

Full description of the Phi-EC2 bacteriophage infecting the phytopathogenic bacteria of the genus Dickeya

Sarah YAMMINE (1,2), Daryna SOKOLOVA (3,4), Lukasz RABALSKI (5), Maciej KOSINSKI (5), Magdalena NARAJCZYK (6), Guy CONDEMINE (7), Stéphane COCIANCICH (1,2) and Robert CZAJKOWSKI (3)

(1) Cirad, UMR PHIM, F-34398 Montpellier, France
(2) PHIM, Université Montpellier, Cirad, INRAe, Institut Agro, IRD, F-34398 Montpellier, France
(3) Laboratory of Biologically Active Compounds, Intercollegiate Faculty of Biotechnology UG and MUG, University of Gdansk, A. Abrahama 58, 80-307 Gdansk, Poland
(4) Department of Biophysics and Radiobiology, Institute of Cell Biology and Genetic Engineering, National Academy of Sciences of Ukraine, Academika Zabolotnoho St. 148, Kiev 03143, Ukraine
(5) Laboratory of Recombinant Vaccines, Intercollegiate Faculty of Biotechnology UG and MUG, University of Gdansk, A. Abrahama 58, 80-307 Gdansk, Poland
(6) Bioimaging Laboratory, Faculty of Biology, University of Gdansk, 80-307 Gdansk, Poland
(7) MAP UMR5240 CNRS-UCBL-INSA Lyon, bâtiment Lwoff, 10 rue Dubois, Campus de la Doua, 69622 Villeurbanne

Bacteriophages (commonly called phages) are natural viruses that target bacteria with, generally, an infection capacity restricted to several strains within a bacterial species (Miroshnikov et al., 2021). Considered as the most abundant living entity on Earth (approximately 10 31), phages are virtually present in all ecological niches harboring bacteria. The pectinolytic genus Dickeya is one of the top ten most damaging plant pathogens based on economic negative impact (Mansfield et al., 2012), and stands as a model for dozens of research teams throughout the world.

Dickeya bacteria are somehow reluctant to transformation by conventional techniques like conjugation or electroporation. Interestingly, generalized phage transduction appears as an effective mechanism to transfer DNA from one Dickeya strain to another. Indeed, in some of our recent studies, we successfully used the generalized transduction technique using the temperate phage Phi-EC2 that was identified forty years ago in Dickeya (Résibois et al., 1984). Intriguingly, the phage Phi-EC2 has been used in several studies of generalized transduction or genome mapping (Schoonejans et al., 1987 ; Hugouvieux-Cotte-Pattat et al., 1989 ; Franza et al., 1991), but nothing more than the scarce content of the 1984 original publication is available to describe this phage.

Here, for the first time, we provide many new data on Dickeya bacteriophage Phi-EC2. We sequenced the phage genome as well as a Dickeya dadantii 3937 lysogenic strain harboring the corresponding prophage. Among other techniques, sequence analyses and electron microscopy allowed us to unravel many features of the Phi-EC2 phage.

References:

Miroshnikov KA, Evseev PV, Lukianova AA, Ignatov AN. 2021. Tailed lytic bacteriophages of soft-rot Pectobacteriaceae. Microorganisms 9:1819. https://doi.org/10.3390/microorganisms9091819

Mansfield J, Genin S, Magori S, Citovsky V, Sriariyanum M, Ronald P, Dow M, Verdier V, Beer SV, Machado MA, Toth I, Salmond G, FosterGD. 2012. Top 10 plant pathogenic bacteria in molecular plant pathology. Mol Plant Pathol 13:614-29. https://doi.org/10.1111/j.1364-3703.2012.00804.x

Résibois A, Colet M, Faelen M, Schoonejans E, Toussaint A. 1984. ϕEC2, a new generalized transducing phage of Erwinia chrysanthemi. Virology 137:102-112. https://doi.org/10.1016/0042-6822(84)90013-8

Schoonejans E, Expert D, Toussaint A. 1987. Characterization and virulence properties of Erwinia chrysanthemi lipopolysaccharide-defective, phi EC2-resistant mutants. J Bacteriol 169:4011-4017. https://doi.org/10.1128/jb.169.9.4011-4017.1987

Hugouvieux-Cotte-Pattat N, Reverchon S, Robert-Baudouy J. 1989. Expanded linkage map of Erwinia chrysanthemi strain 3937. Mol Microbiol 3:573-81. https://doi.org/10.1111/j.1365-2958.1989.tb00204.x

Franza T, Enard C, van Gijsegem F, Expert D. 1991. Genetic analysis of the Erwinia chrysanthemi 3937 chrysobactin iron-transport system: characterization of a gene cluster involved in uptake and biosynthetic pathways. Mol Microbiol 5:1319-29. https://doi.org/10.1111/j.1365-2958.1991.tb00778.x

Are You Sure You Are Working with the Right Bacterial Strain ? The Case of Dickeya solani Type Strain IPO 2222

Quentin DUBOIS (1), Typhaine BRUAL (1), Jérémy CIGNA (2), Romain MERCIER (3), Laetitia ATTAIECH (4), Agnès RODRIGUE (1), Erwan GUEGUEN (1).

(1) Univ Lyon, Université Claude Bernard Lyon1, CNRS, INSA Lyon, UMR5240 MAP, F-69622, LYON, France.

(2) Paris-Saclay University, CNRS, CEA, Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France.

(3) Laboratoire de Chimie Bactérienne (LCB), Institut de Microbiologie de la Méditerranée (IMM), CNRS - Aix-Marseille Université UMR7283, Marseille, France.

(4) CIRI, Centre International de Recherche en Infectiologie, Team “Horizontal gene transfer in bacterial pathogens” (Horigene), Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, Ecole Normale Supérieure de Lyon, Univ Lyon, 69100, Villeurbanne, France

Dickeya solani is a highly aggressive pathogen of potato crops. The type strain IPO 2222, originally isolated from a diseased potato plant in the Netherlands, was the first strain to be sequenced and has been widely used as a model in research laboratories across Europe. Despite its importance as a reference strain, IPO 2222 is not the most virulent strain. Other strains isolated from different countries exhibit significantly higher pathogenicity. For a long time, the reason for this weaker pathogenic potential in IPO 2222 remained unclear, as all known virulence genes are present. The variation between strains appears to result from differences in gene expression regulation.

Recently, two European laboratories published contradictory findings on a new phenotypic trait of IPO 2222. These conflicting results prompted us to investigate further. We discovered that two distinct IPO 2222 strains are circulating in European laboratories, differing by a single point mutation in a regulatory gene. Our findings demonstrate that this mutation leads to the inactivation of the gene, explaining the phenotypic differences between the two strains. We have accordingly renamed them IPO 2222a and IPO 2222b.

Our study highlights the critical importance of sequencing laboratory model strains and comparing the obtained genomes with those available in public databases to ensure experimental consistency and accuracy.

References:

Role and regulation of secondary metabolite genes in symbiont of leaf symbiosis

Erin HILLAIRET (1), Aurélien CARLIER

(1) LIPME - INRAE Toulouse

Natural products (NPs) are a diverse group of bioactive compounds with important roles in medicine and agriculture, often produced by microorganisms such as bacteria and fungi. These NPs are typically synthesized by specialized gene clusters, known as Biosynthetic Gene Clusters (BGCs), which are often tightly regulated, leading to challenges in fully exploiting their potential. In Dioscorea sansibarensis, a unique symbiosis exists with the bacterium Orrella dioscoreae, where the bacterium inhabits the plant's leaf glands and is inherited through aerial bulbils. Recent genomic studies revealed three key BGCs in O. dioscoreae (smp1, smp2, and opk), with the opk clusters showing homology to genes involved in bioactive compound production. Furthermore the genes belonging to these clusters are among the most highly expressed in the leaf gland. Transcripts of smp and opk have make up about 30% of all mRNA reads.

Our findings indicate that quorum sensing (QS) plays a central role in regulating the smp cluster. This tight regulation suggests that QS may serve to finely tune the production of bioactive compounds, ensuring their expression occurs only under specific conditions that likely reflect ecological pressures. Understanding the molecular mechanisms driving this regulation is essential for deciphering how microbial secondary metabolism is orchestrated in symbiotic relationships, and what functions it might play. These insights could have broader implications for the discovery of new NPs and for understanding the evolutionary advantages of maintaining strict control over BGC expression in microbial symbioses.

References:

Acar, T., Moreau, S., Coen, O., De Meyer, F., Leroux, O., Beaumel, M., Wilkin, P., & Carlier, A. (2022). Motility-Independent Vertical Transmission of Bacteria in Leaf Symbiosis. mBio, 13(5), e0103322. https://doi.org/10.1128/mbio.01033-22

De Meyer, F., Danneels, B., Acar, T., Rasolomampianina, R., Rajaonah, M. T., Jeannoda, V., & Carlier, A. (2019). Adaptations and evolution of a heritable leaf nodule symbiosis between Dioscorea sansibarensis and Orrella dioscoreae. The ISME Journal, 13(7), 1831-1844. https://doi.org/10.1038/s41396-019-0398-8

Impact of 3D Chromosome Conformation on Gene Expression and Virulence in Dickeya

Oyut DAGVA*(1), Jessica BAUDE*(1), Maëlys DELOUIS*(2), Sylvie REVERCHON (1), Pauline LARROUS (2), Cécile RIBOT (1), Agnès THIERRY (2), Florence HOMMAIS (1), Romain KOZUL (2), Sam MEYER (1), Zahar HAICHAR (1), Axel COURNAC (2)*, William NASSER (1)*

(1) INSA-Lyon, CNRS, UMR5240, Microbiologie, Adaptation, Pathogénie, Univ. Lyon, Université Lyon 1, 69622 Villeurbanne, France
(2) Institut Pasteur, CNRS UMR 3525, Université Paris Cité, Unité Régulation Spatiale des Génomes, 75015 Paris, France
* These authors contributed equally

Bacterial adaptation to environmental changes requires a rapid reorganization of expression pattern of their genome. This adaptive response, which is critical for both bacterial survival and pathogenicity, is mediated by specific transcription factors, DNA supercoiling and global regulators represented by abundant nucleoid-associated-proteins (NAPs) (Reverchon et al., 2021; Nasser et al., 2001; Lioy et al., 2018).

Our group demonstrated the correlation between gene expression and the subcellular localization of loci in response to environmental changes in the necrotrophic plant pathogen Dickeya. Loci located in domains activated by a specific stress are specifically repositioned from the center to the periphery of the nucleoid while loci in domains repressed by the same stress move from the periphery to the center of the nucleoid. These relocations align with increased RNA polymerase accessibility at the nucleoid surface when a locus is activated (Stracy et al., 2015). We aim to further investigate the molecular mechanisms involved in the differential chromosome folding under changing environmental conditions and the repercussion on gene-expression profiles. Our recent HiC and transcriptomic data showed a clear link between gene expression and the spatial organization of the genome of Dickeya dadantii. Under osmotic stress conditions, a strongly repressed domain is indeed correlated with the appearance of a region with high physical contacts, which likely reflects a particular folding of this region, making it less accessible to RNA polymerase or suggesting a specific NAP occupancy.

Dickeya is a major pathogen causing soft-rot disease in a wide range of plant hosts, including economically important vegetables such as potatoes, maize, and rice (Reverchon and Nasser, 2013; Blin et al., 2021). In this context, our work aims to capture a comprehensive view of bacterial chromosome dynamics and its impact on the coordination of adaptive processes and virulence functions.

References:

PM 7/24 (5) (2023), Xylella fastidiosa. EPPO Bulletin, 53: Blin, P., Robic, K., Khayi, S., Cigna, J., Munier, E., Dewaegeneire, P., et al. (2021). Pattern and causes of the establishment of the invasive bacterial potato pathogen Dickeya solani and of the maintenance of the resident pathogen D. dianthicola. Molecular Ecology 30, 608–624. doi: 10.1111/mec.15751

Lioy, V. S., Cournac, A., Marbouty, M., Duigou, S., Mozziconacci, J., Espéli, O., et al. (2018). Multiscale Structuring of the E. coli Chromosome by Nucleoid-Associated and Condensin Proteins. Cell 172, 771-783.e18. doi: 10.1016/j.cell.2017.12.027

Nasser, W., Faelen, M., Hugouvieux-Cotte-Pattat, N., and Reverchon, S. (2001). Role of the Nucleoid-Associated Protein H-NS in the Synthesis of Virulence Factors in the Phytopathogenic Bacterium Erwinia chrysanthemi. MPMI 14, 10–20. doi: 10.1094/MPMI.2001.14.1.10

Reverchon, S., Meyer, S., Forquet, R., Hommais, F., Muskhelishvili, G., and Nasser, W. (2021). The nucleoid-associated protein IHF acts as a 'transcriptional domainin' protein coordinating the bacterial virulence traits with global transcription. Nucleic Acids Research 49, 776–790. doi: 10.1093/nar/gkaa1227

Reverchon, S., and Nasser, W. (2013). Dickeya ecology, environment sensing and regulation of virulence programme. Environ Microbiol Rep 5, 622–636. doi: 10.1111/1758-2229.12073

Stracy, M., Lesterlin, C., Garza de Leon, F., Uphoff, S., Zawadzki, P., and Kapanidis, A. N. (2015). Live-cell superresolution microscopy reveals the organization of RNA polymerase in the bacterial nucleoid. Proceedings of the National Academy of Sciences 112, E4390–E4399. doi: 10.1073/pnas.1507592112

The beneficial effect of Enterobacter sp. SA187 on the development of Arabidopsis thaliana under limited nitrogen conditions

Amina ILYAS, Caroline MAUVE, Michael HODGES, Axel de ZELICOURT

Université Paris-Saclay, CNRS, INRAE, Université Paris Cité, Université d'Evry, Institute of Plant Sciences Paris-Saclay (IPS2), Bât. 630, Avenue des Sciences, 91190 Gif-sur-Yvette, France

Nitrogen (N) is an essential yet often limited nutrient for plants. Insufficient N availability negatively impacts plant growth and crop yield, typically mitigated by the widespread use of N-fertilizers. However, synthetic N-fertilizer application threatens agricultural sustainability, contributing to water pollution and global climate change. Thus, finding new ways to improve or maintain crop yields under low N conditions is crucial. We are studying how Enterobacter sp. SA187 (SA187), a bacterium promoting abiotic stress tolerance (de Zélicourt et al., 2018; Shekawat et al., 2021), affects Arabidopsis thaliana growth under varying N concentrations (0.2-10 mM) and sources (NO3- or NH4NO3-). SA187 consistently improved plant biomass and root architecture under all low N conditions tested, regardless of the N source. Interestingly, the beneficial effect of SA187 increased with decreasing N concentration. Our ongoing research employs plant transcriptomics, metabolomics, 15N-labeling, elemental analyses, enzymatic activities, and assessments of plant nitrate and ammonium levels, as well as N allocation, to elucidate the mechanisms involved. Transcriptomics analyses revealed the possible involvement of the ethylene signaling pathway and specific nitrate transporters in SA187-mediated growth promotion. Ethylene-insensitive (ein2-1) and high-affinity nitrate transporter (nrt2.5 x nrt2.6) mutants indicated that ethylene signaling and nitrate transport are involved in the observed beneficial effect. These preliminary findings suggest that establishing an SA187-plant interaction could be a promising strategy to maintain plant growth and development under limited N conditions, thus reducing N-fertilizer use. By unraveling the physiological and molecular mechanisms of this interaction, we aim to provide novel insights into the role of non-N fixing bacteria in plant nutrition and productivity under low N conditions, ultimately contributing to sustainable agricultural practices.

References:

de Zélicourt A., Synek L., Saad M.M., Alzubaidy H., Jalal R., Xie Y., Andrés-Barrao C., Rolli E., Guerard F., Mariappan K.G et al.(2018). Ethylene induced plant stress tolerance by Enterobacter sp.SA187 is mediated by 2-keto-4-methylthiobutyric acid production.PLOS Genetics 14(3), e1007273.

Shekhawat, K., Saad, M.M., Sheikh, A., Mariappan K.G., Al-Mahmoudi H., Abdulhakim F., Eida A.A., Jalal R., Masmoudi K., & Hirt H. (2021). Root endophyte induced plant thermotolerance by constitutive chromatin modification at heat stress memory gene loci. EMBO Reports, 22(1), e51068.

The type III-secreted effector HopT1-1 hijacks the function of a novel host susceptibility factor to suppress microRNA activity and cause disease

Marc PRZYSZSZYPKOWSKI (1), Magali CHARVIN (1), Eve BOUTRON (1), Laetitia SCALISCI (1), Alvaro Pérez (2) and Lionel NAVARRO (1) & Gersende LEPERE (1)

1- IBENS-46 rue d'Ulm 75005 Paris

2- IRD-PHIM 911, Av. Agropolis 34394 Montpellier

The Arabidopsis microRNA (miRNA) pathway is crutial for basal immunity against Pseudomonas syringae pv. tomato strain DC3000 (Pto DC3000). As a counter-defense strategy, Pto DC3000 has evolved type III effectors that suppress different steps of the miRNA pathway to enable disease. We have shown that the HopT1-1 effector suppresses both miRNA activity and Pattern-Triggered Immunity (PTI). HopT1-1 is additionally sensed by host cells, which results in Effector-Triggered Immunity (ETI). Both HopT1-1-triggered RNA silencing/PTI suppression and ETI activation are dependent on the ability of HopT1-1 to physically interact with the central miRNA factor Argonaute 1 (AGO1) through conserved glycine/tryptophan motifs. In order to further understand how HopT1-1 suppresses miRNA activity and cause disease, we have conducted a forward genetic screen at the level of stomata. So far, 11 independent mutants were isolated and are currently under characterization. These Arabidopsis mutants restore miRNA activity in the presence of HopT1-1. I will report on the characterization of Repressor of Bacterial Silencing suppressor 1 (RBS1), which encodes a novel host susceptibility factor whose inactivation impairs the ability of HopT1-1 to suppress miRNA activity/PTI and to mount ETI. Our findings suggest that HopT1-1 has evolved to hijack RBS1 function at the level of stomata to suppress miRNA activity and cause disease. Given the fact that RBS1 represents a functionnaly relevant target of HopT1-1, we will determine whether HopT1-1 could physically interact with RBS1. Moreover, our in silico analyses revealed that RBS1 exhibits a high probality to undergo liquid-liquid phase separation. Therefore we will determine whether it could form condensates in in vitro and in vivo conditions and if this feature is revelant for AGO1-dependent PTI and RNA silencing functions. Overall, these analyses should provide novel insights into the mechanisms by which RBS1 functions at the AGO1-miRISC level.

References:

- Role of stomata in plant innate immunity and foliar bacterial diseases. M. Melotto, W. Underwood and S. Y. He. Annu Rev Phytopathol 2008 Vol. 46 Pages 101-22

- Suppression of the microRNA pathway by bacterial effector proteins.L. Navarro, F. Jay, K. Nomura, S. Y. He and O. Voinnet. Science 2008 Vol. 321 Issue 5891 Pages 964-7

- A bacterial GW-effector directly targets Arabidopsis Argonaute 1 to suppress PAMP-triggered immunity and cause disease. Thiébeauld O et al.; bioRXiv 2023. https://doi.org/10.1101/215590

Two histone-modifying enzymes reprogram symbiotic nodulation depending on nitrogen availability

Naïs DAVIDDI (1,2), Rim BRIK-CHAOUCHE (1,2), Xiaoning HE (1,2), Moussa BENHAMED (1,2), Florian FRUGIER (1,2), Camille FONOUNI-FARDE (1,2)

(1) Université Paris-Saclay, CNRS, INRAE, Université d'Evry, Institute of Plant Sciences Paris-Saclay (IPS2), 91190, Gif sur Yvette, France.

(2) Université Paris Cité, CNRS, INRAE, Institute of Plant Sciences Paris-Saclay (IPS2), 91190, Gif sur Yvette, France.

Under low-nitrogen (N) conditions, legume plants associate with soil bacteria referred to as rhizobia, to form symbiotic root nodules in which atmospheric N_2 is fixed and assimilated by the plant. To achieve maximum gains, nodule number and N_2-fixation activity are precisely controlled by the host plant through a combination of local and systemic regulatory pathways that integrate shoot and root signals. Among them, CEP signaling peptides systemically promote nodulation under low-N through the CRA2 receptor acting in shoots. The molecular effectors and targets of the CEP/CRA2 pathway remain however poorly documented despite its tremendous impact on the adaptation of root system architecture to low-N. Among the CRA2 systemic targets identified in the model legume *Medicago truncatula* using transcriptomic analyses, two histone-modifying enzymes were retrieved: the histone deacetylase MtHDT2 (HISTONE DEACETYLASE 2) and the histone methyltransferase MtATX3.1 (ARABIDOPSIS TRITHORAX 3.1). We showed that MtHDT2 and MtATX3.1 regulate the establishment of nodulation by controlling bacterial infection during the early stages of nodulation. In addition, we showed that both MtHDT2 and MtATX3.1 regulate the transcription of key genes involved in the regulation of N-responses and symbiotic nodulation. More precisely, we identified two MtHDT2 direct target genes acting in roots downstream CRA2 and involved in root development and/or nodulation in *M. truncatula*, namely *NIN-LIKE PROTEIN 1* (*NLP1*) and *basic helix-loop-helix-658* (*bHLH-658*).

References:

Roy S, Liu W, Nandety RS, et al. Celebrating 20 Years of Genetic Discoveries in Legume Nodulation and Symbiotic Nitrogen Fixation. Plant Cell. 2020;32(1):15-41.

Gautrat P, Laffont C, Frugier F, Ruffel S. Nitrogen Systemic Signaling: From Symbiotic Nodulation to Root Acquisition. Trends Plant Sci. 2021;26(4):392-406.

Luo Z, Moreau C, Wang J, Frugier F, Xie F. NLP1 binds the CEP1 signalling peptide promoter to repress its expression in response to nitrate. New Phytol. 2022;234(5):1547-1552.

Zahaf O, Blanchet S, de Zélicourt A, et al. Comparative transcriptomic analysis of salt adaptation in roots of contrasting Medicago truncatula genotypes. Mol Plant. 2012;5(5):1068-1081.

The Arabidopsis root adaptative response to drought is unlikely to depend on the ABA signalling pathway when it is colonized by beneficial rhizobacteria

Hugo DUPUY (1), Lilou HENRENT (1), Philippe NACRY (1), Claire PRIGENT-COMBARET (2), Guilhem DESBROSSES (1)

(1) UMR IPSiM, Univ Montpellier, CNRS, INRAE, Institut Agro, Montpellier, France

(2) UMR CNRS 5557 Ecologie Microbienne - University Claude Bernard Lyon 1, Villeurbanne, France

The root growth adaption to drought is an essential trait for plants to achieve their full developmental program. To better understand the underlying mechanisms, many studies have been conducted on Arabidopsis thaliana plantlets grown in vitro (1). They show that root developpment is modified as soon as drought is percieved (2) and that this process involves the ABA signalling pathway (3). But there are other stresses that alter Arabidopsis root development included the presence of beneficial microbes in the rhizosphere (3). It opens an important question : are biotic and abiotic related signalling pathways interacting with each other when they are simultaneously triggered ?

To explore that question, we grew Arabidosis plants in vitro under a drought stress or not and, in presence or in absence of different beneficial bacteria strains. Eight days after the treatment, we measured the root growth.

We confirm that drought and the colonisation of the root by some of the selected strains do independently inhibit root growth. Yet, when the two stresses are combined, some strains do aleviate drought-dependent root growth inhibition while others not.

We also explored the involvement of the ABA signalling pathway using a plant specific reporter line. As expected, drought activates the ABA signalling pathway in the root, but remain activated in presence of beneficial microbes that restore root growth. Under drought condition, root growth restoration by some beneficial microbes would be a process independent from the ABA signalling pathway.

References:

(1) Gupta A, Rico-Medina A, Caño-Delgado AI. The physiology of plant responses to drought. doi: 10.1126/science.aaz7614.

(2) Smith S, De Smet I. Root system architecture: insights from Arabidopsis and cereal crops. doi: 10.1098/rstb.2011.0234.

(3) Hong JH, Seah SW, Xu J. The root of ABA action in environmental stress response.

doi: 10.1007/s00299-013-1439-9. .

(4)Verbon EH, Liberman LM. Beneficial Microbes Affect Endogenous Mechanisms Controlling Root Development.

doi: 10.1016/j.tplants.2016.01.013.

How plants select their bacterial partners: an Experimental Evolution on plant Beneficial Rhizobacteria

DORE Jeanne (1), DESBROSSES Guilhem (2), MULLER Daniel (1) and PRIGENT-COMBARET Claire (1)

(1) UMR CNRS 5557 Ecologie Microbienne, Univ Claude Bernard Lyon 1, Villeurbanne, France

(2) UMR IPSiM, Univ Montpellier, CNRS, INRAE, Institut Agro, Montpellier, France

Plant interacts with multiple microbial players, together constituting the plant holobiont. At the level of the plant roots, a specific rhizospheric microbiota is shaped mostly under the influence of exudates. Some bacteria within this rhizomicrobiota promote plant growth and protect it against abiotic and biotic stresses. However, most molecular studies on plant-microbe cooperation are performed on one plant inoculated by one bacterium, and despite the wealth of knowledge brought by these studies, they failed to predict how a strain would protect a plant grown in the field. This limitation likely arises because we do not know how plant-beneficial bacteria may interplay with other rhizomicrobiota members and the plant itself. To explore the adaptive mechanisms involved in plant-bacteria cooperation, our group has developed an experimental evolution approach, carried out on a synthetic community (SynCom), made up of 10 cooperative bacteria belonging to distinct Pseudomonas species, evolving for 400 generations in presence and absence of plants. Through metabarcoding analysis, we tracked changes in the SynCom, observing rapid shifts in community composition and a strong plant influence in directing the assembly of Pseudomonas populations. We are currently investigating the genomic and phenotypic changes in these evolved populations, and how both the evolved and ancestral SynComs may impact plant physiology.

References:

The active DNA demethylase ROS1 shapes antibacterial immune responsiveness by facilitating DNA binding of WRKY transcription factors

Thierry HALTER (1), Magali CHARVIN (1), Louna DE OLIVEIRA (1), Alvaro PEREZ QUINTERO (1), Romain BLANC-MATHIEU (2), Pierre BADUEL (1), Pierre BARRAUD (3), Olivier LOUDET (4), Vincent COLOT (1), Francois PARCY (2) and Lionel NAVARRO (1)

(1) Institut de Biologie de l'Ecole Normale Supérieure (IBENS), Paris

(2) Laboratoire de Physiologie Cellulaire et Végétale (LPCV), Grenoble

(3) Institut de Biologie Physico-Chimique (IBPC), Paris

(4) Institut Jean-Pierre Bourgin (IJPB), Versailles

DNA methylation is an epigenetic mark that silences transposable elements (TEs) and some genes carrying repeats in their vicinity. DNA methylation machineries negatively regulate resistance against Pto DC3000. By contrast, DNA demethylation by ROS1 positively regulates plant defense against this bacterium. Arabidopsis immune-responsive genes that are positively controlled by ROS1 during elicitation with flg22 were identified and, among them, we retrieved immune receptors such as the surface receptors RLP43 and RLP53, and the NLR RMG1. Importantly, siRNA-directed remethylation at RLP43, RLP53 and RMG1 promoter regions abolishes flg22-triggered induction of these genes and reduce antibacterial resistance. It suggests that demethylation at the promoters of these genes is required for their proper transcriptional activation during plant immunity. We found the WRKY TF family to be over-represented at ROS1-targeted promoter regions. Specific DAP-qPCR at RLP43 promoter demonstrated that erasure of methylation by ROS1 is required for WRKYs binding. Using crystal structure of WRKY40 and DAP-seq dataset, we show that methylation of a single cytosine inside the W-box is sufficient to inhibit WRKY-binding onto DNA. Finally, we investigated natural DNA methylation variation at ROS1-regulated regions and showed that epialleles at these regions, including at the promoter of RLP43 and RLP53, are widespread in nature. Interestingly, flg22-induced transcriptional activation was impaired in accessions carrying hypermethylated epialleles of RLP43 and RLP53, mimicking ros1 mutants. Overall, these studies supports a direct role for active demethylation in facilitating WRKY TF DNA binding at the promoter of defense genes, thereby ensuring their pervasive induction upon pathogen detection. We provide evidence that ROS1 activity can vary at specific loci during evolution to fine-tune gene expression, responsiveness, and possibly promote adaptation to specific environment.

References:

Charvin, M., Halter, T., Blanc-Mathieu, R., Barraud, P., Aumont-Nicaise, M., Parcy, F., Navarro, L., 2023. Single-cytosine methylation at W-boxes repels binding of WRKY transcription factors through steric hindrance. Plant Physiology 192, 77–84. https://doi.org/10.1093/plphys/kiad069

Halter, T., Wang, J., Amesefe, D., Lastrucci, E., Charvin, M., Singla Rastogi, M., Navarro, L., 2021. The Arabidopsis active demethylase ROS1 cis-regulates defence genes by erasing DNA methylation at promoter-regulatory regions. Elife 10, e62994. https://doi.org/10.7554/eLife.62994

Yu, A., Lepère, G., Jay, F., Wang, J., Bapaume, L., Wang, Y., Abraham, A.-L., Penterman, J., Fischer, R.L., Voinnet, O., Navarro, L., 2013. Dynamics and biological relevance of DNA demethylation in Arabidopsis antibacterial defense. Proc. Natl. Acad. Sci. U.S.A. 110, 2389–2394. https://doi.org/10.1073/pnas.1211757110.

Untangling the complex regulation of plant polysaccharides degradation by *Streptomyces coelicolor*

Augustin RIGOLET(1), Rachel STUIJ(1), Silvia RIBEIRO MONTEIRO(2), Yasmine KERDEL(2), Sébastien RIGALI(2), Gilles P. VAN WEZEL(1)

(1) Institute of Biology, Leiden University, Netherlands
(2) Centre d'ingénierie des protéines (CIP), University of Liège, Belgium
a.rigolet@biology.leidenuniv.nl

The soil, and in particular the rhizosphere, are ecological hotspots marked by nutrient scarcity. There, most carbon originates from plants that secrete root exudates and synthesize complex polysaccharides such as cellulose, hemicelluloses, starch, pectin or callose which contribute to the soil organic matter. In this nutrient-limited environment, the saprophytic streptomycetes have evolved an extensive extracellular biology to grow on such complex polysaccharides. For instance, the model organism *Streptomyces coelicolor* encodes in its genome 819 predicted secreted protein, including dozens of predicted glycosyl hydrolases and 10 polysaccharide lyases[1].

The intracellular regulation mechanisms governing the degradation of complex polysaccharides in *Streptomyces* appear central for its development and ecology. For instance, the regulation of chitin/chitosan degradation is linked to antibiotic production and programmed cell death via DasR[2]. Meanwhile, the cellobiose responsive regulator CebR plays a central role in controlling the pathogenicity of *Streptomyces scabies,* the causative agent of the potato scab disease[3]. Thus, beyond simple nutrient acquisition, *Streptomyces*' ability to degrade complex polysaccharides – and the involved regulation- may also support additional ecological roles, such as facilitating interactions with plant roots and promoting mutualistic or associative relationships within the rhizosphere, as reported in other root-associated genera[4–7]. However, beyond these examples, the regulation of complex polysaccharides degradation remains poorly understood.

In this study, we aim to iron out the regulation of the main polysaccharides present in soil. We generated *in silico* predictions of the transcription factors (TFs) likely controlling the degradation and uptake of these polysaccharides. This uncovered numerous LacI-type regulators dedicated to the degradation of complex sugars. Specifically, we propose the TFs responsible for the breakdown of pectin, callose (a plant defense polysaccharide), alginate, agar and mannan and fungal mannan-like polysaccharides, and we untangled the intricated regulation of arabino-galactan degradation. We are currently running experiment to test those predictions.

References:

1. doi:10.1111/j.1574-6976.2009.00206.x
2. doi:10.1038/embor.2008.83
3. doi:10.1021/acs.jproteome.8b00528
4. doi:10.1073/pnas.1218984110
5. doi:10.1111/1758-2229.12286
6. doi:10.1016/j.isci.2023.107925
7. doi:10.1128/mBio.01774-21

Keywords: Streptomyces, polysaccharide, regulation, complex carbon sources

The Ralstonia solanacearum species complex in the age of epidemiology: exploration of its molecular diversity and population structure

Gilles CELLIER (1); Yann PECRIX (2)

Gilles CELLIER (1); Yann PECRIX (2)

1 Plant Health Laboratory, Anses, Saint Pierre, Reunion Island

2 CIRAD, UMR Peuplements végétaux et bioagresseurs en milieu tropical, Saint Pierre, Reunion Island

gilles.cellier@anses.fr

As long-distance traveling of human beings and movement of goods drastically increase over time in relation to globalization of trade and exchanges, and so is the spread of bacterial pathogens and associated infectious diseases across the globe. Key for improved disease control lies into acquiring a thorough knowledge on factors shaping pathogen populations at fine scales and how they interact with their environment.

In order to show much more clearly how infectious agents are spreading and evolving other than genetic drift alone, phylogenetic and epidemiological techniques are often use. Bacterial lineage-centered molecular genotyping techniques, such as multilocus variable number of tandem repeats analysis (MLVA). They are of interest especially when they provide high throughput, a sound phylogenetic signal, and a resolution fitting the spatiotemporal scale investigated. In the case of complex plant pathogens, such as the Ralstonia solanacearum species complex (RSSC), several studies achieved molecular characterization of outbreak strains during the last decade, and brought light on its epidemiology.

Nevertheless, selecting proper genetic marker and analytical algorithm is vital to apply molecular genetics in a given biological population. Because phylogenetic analysis is inexpensive, especially when sequence data are already available, it is important for molecular epidemiologists to understand, to correctly apply, and to correctly interpret phylogenies and phylogenetic methods. We will present last epidemiological results produced within the framework of the phylotype I of the Ralstonia solanacearum species complex, which revealed the need for a curated phylogenetic database, deep sampling for discovering epidemiological impacting lineages, and the adoption of complementary multiscale analyses from gene to genome in order to apprehend the evolution complexity as a whole.

References:

Pectobacterium versatile beta-lactamase, a common good of the soft rot Pectobacteriaceae (SRP) species complex

Lorang Camille, Canto Pierre Yves, Dutey Elena, Pédron Jacques and Barny Marie-Anne (1)

(1) IEES - CNRS Paris

The wealth of data on clinical antibiotics, the evolution of associated resistance mechanisms and their spread through mobile genetic elements, contrasts with the lack of knowledge about the role of antibiotic in microbial ecosystems in the absence of clinical antibiotic pressure. The soft rot Pectobacteriaceae (SRP) species complex, comprise more than 40 different bacterial species and is responsible for severe plant rotting in many plant species. Within this complex, most Pectobacterium versatile strains harbor a β-lactamase, called BlaPEC1. The aim of the present work was to analyze the role of BlaPEC1 in the context of plant infection in the absence of clinical antibiotic pressure. To this end, we constructed blaPEC1-deleted strains in two different P. versatile strains and compared them with their wild-type counterparts in vitro and in potato tuber mono-infection or mixed infection with different SRP species and strains. In vitro, BlaPEC1 enables P. versatile to resist ampicillin or the carbapenem produced by Pectobacterium brasiliense. In potato tuber mono-infections, blaPEC1-deleted strains are unaffected in virulence and fitness. However, BlaPEC1 was found to be required for the coexistence of P. versatile with carbapenem-producing P. brasiliense. Furthermore, the P. versatile strains producing BlaPEC1 allow the coexistence of Pectobacterium strains sensitive to P. brasiliense produced carbapenem, both in vitro and in planta. This effect is spatially dependent and is observed even when the BlaPEC1-expressing strain is a minority within the symptom. These results indicate that Blapec1 exerts a true β-lactamase function that is efficient during the infection process. Blapec1 can therefore be considered a public good of the SRP species complex, allowing the maintenance of strain diversity within this species complex.

References:

Royer G, Dixit Z, Pédron J, Pierrat G, Demontant V, Berçot B, Rodriguez C, Barny MA, Jacquier H, and Woerther PL. (2022) Genetic and phenotypic study of the Pectobacterium versatile beta-lactamase, the enzyme most similar to the plasmid-encoded TEM-1" Applied and Environmental Microbiology #AEM00220-22R1)

Expansion of Bacterial leaf Blight Disease of Rice Caused by Xanthomonas oryzae pv. oryzae in East Africa

Brottier L (1)†, Blasco C (1)†, Auguy F (1), Nganga E (3), Eyokia M (4) , Hashim I (2), Mihayo R (2), Leonard H (2), Lihepanyama D (2), Mkuya M (2), Onaga G (5), Bigiramana J (4), Dereeper A (1), Cunnac S (1), Neema C (1), Hutin M (1), Szurek B (1)

(1) IRD, Institut de Recherche pour le Développement UMR PHIM, Plant Health Institute of Montpellier Univ Montpellier, CIRAD, INRAE, Institut Agro, IRD, Montpellier, France; (2) Tanzania Agricultural Research Institute, TARI Ifakara Centre, Private Bag, Ifakara Tanzania;(3) International Rice Research Institute (IRRI) - Africa Regional Office, P.O. Box 30709 Nairobi 00100, Kenya; (4) International Rice Research Institute (IRRI) -Burundi Office, Mutanga Campus of Burundi University, Avenue de l'UNESCO nr 2, Bujumbura-Burundi; (5) Africa Rice Center (AfricaRice) 01 BP 2551 Bouake 01, Côte d'Ivoire.

† Brottier L and Blasco C contributed equally to this work

Bacterial leaf blight (BLB), caused by the bacterium Xanthomonas oryzae pv. oryzae (Xoo), is a devastating disease of rice crops in many countries, causing yield losses of 20-30% and up to 50% (Mew 1987). BLB was first reported in Japan in 1884 and is now prevalent in most of the rice-producing countries in Asia and West African countries.

Recently, BLB also appears to be spreading in East Africa with reports in Tanzania in 2019 (Schepler-Luu et al., 2023). Several sampling surveys carried out in last years have demonstrated the spread of this disease in various regions of Tanzania, right up to the crossing of Tanzania's frontiers with Kenya and in Uganda.

The Xoo strains isolated from these surveys have been characterized by genotyping using multiple locus VNTR analysis (MLVA), and representative strains have been sequenced using nanopore technology. Our results suggest that Xoo has been introduced once in the region of Morogoro, potentially owed to the import of contaminated seed material originating from Asia. With the aim of searching for resistance sources, NILs with anti-Xoo Xa resistance genes were screened, resulting in the identification of three genes of interest. This work will serve as a basis for future rice breeding for BLB resistance efforts in East-African countries.

References:

Mew, T. W. Current Status and Future Prospects of Research on Bacterial Blight of Rice. Annual Review of Phytopathology 25, 359–382 (1987).

Schepler-Luu, V. et al. Genome editing of an African elite rice variety confers resistance against endemic and emerging Xanthomonas oryzae pv. oryzae strains. eLife 12, (2023).

Genomic diversity and aggressiveness study of the emerging pectinolytic bacteria species P. brasiliense in potato fields

Gwendoline JONCOUR (1,2), Angélique LAURENT (1,3), Denis FAURE (2), Jérémy CIGNA (1,2)

(1) FN3PT-inov3PT, 75008 Paris
(2) CNRS-I2BC, 1 avenue de la terrasse, 91198 Gif-sur-Yvette
(3) IGEPP, Agrocampus Ouest, INRAE, Université de Rennes 1, 35653 Le Rheu

Blackleg causes high economic losses for the seed potato industry worldwide. The disease is caused by bacteria belonging to the genera Pectobacterium or Dickeya. The number of species belonging to these two genera has increased from 12 to 33 described species since 2016. P. brasiliense is one of this species. Absent around ten years ago, P. brasiliense has become predominant in the blackleg symptoms collected from potato fields.

Furthermore, this species is able to infect a lot of vegetable hosts in differents environments, possibly explained by a high genetic diversity level among P. brasiliense genomes. However, the link between detection in seed potato before planting and expression in field has not been established. The objective of the study is to identify which genetic cluster of P. brasiliense are present in potato environment and which ones have an impact on potato production.

In this goal, 60 strains isolated between 2007 and 2023 were selected regarding their dnaX barcod diversity and the genomes of 12 of them were sequenced with nanopore oxford technology to compare the genomes and identify the different clusters. Moreover, tuber maceration test and greenhouse assay were carried out to characterize the aggressiveness of each strains and each clusters. The results showed 3 differents genomic clusters and a significative difference of aggressiveness between clusters. Specific tools will be designed for the most impacting clusters.

References:

What do Arsenophonus and Xylella have in common? Comparative genomics reveals horizontal gene transfers implicated in the emergence of phytopathogenic Arsenophonus strains

Mathieu MAHILLON (1,2), Christophe DEBONNEVILLE (1), Raphael GROUX (1), David ROQUIS (3), Justine BRODARD (1), Franco FAORO (4), Xavier FOISSAC (5), Olivier SCHUMPP (1), Jessica DITTMER (4,6)

1) Research group Virology, Bacteriology and Phytoplasmology, Agroscope, Nyon, Switzerland
2) Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Belgium
3) Haute école du paysage, d'ingénierie et d'architecture de Genève, Switzerland
4) Dipartimento di Scienze agrarie e ambientali, Università degli Studi di Milano, Italy
5) UMR 1332 Biologie du Fruit et Pathologie, Université de Bordeaux, INRAE, Bordeaux, France
6) Université d'Angers, Institut Agro, INRAE, IRHS, SFR Quasav, Angers, France

Numerous bacterial pathogens infecting the plant vasculature are transmitted by insect vectors, necessitating adaptations to two very different hosts. This bi-phasic lifestyle has evolved in diverse taxa via distinct ecological routes, i.e. among initially plant-associated ("plant-first") or insect-associated ("insect-first") bacteria. The genus Arsenophonus is an example for the latter, since the phloem pathogens 'Candidatus Arsenophonus phytopathogenicus' (Ap) and 'Ca. Phlomobacter fragariae' (Pf) have recently evolved from insect endosymbionts. However, the genetic mechanisms underlying this transition have not yet been elucidated. To fill this gap, we assembled the genomes of both strains from insect metagenomes. Functional and phylogenomic analyses indicate that Ap and Pf are similar in size and functional repertoire, despite belonging to different species. Strikingly, we identified a set of orthologous genes present only in Ap and Pf and absent from all other Arsenophonus strains. In particular, both strains share putative plant cell-wall degrading enzymes as well as a set of cysteine peptidases related to xylellain, a papain-like peptidase first described in Xylella fastidiosa which has close homologs in diverse Pseudomonadota infecting the plant vasculature. High expression in planta or in insecta was demonstrated for several of these shared orthologs, further supporting specific roles during phloem or insect colonization. Hence, this work provides evidence that phytopathogenicity emerged twice in Arsenophonus via a limited number of horizontal gene transfer (HGT) events. This work opens new questions regarding the role of xylellain-like peptidases in bacteria-plant interactions and the potential routes for HGT between bacteria with different ecological niches.

References:

Mahillon et al. (2024). From insect endosymbiont to phloem colonizer: comparative genomics unveils the lifestyle transition of phytopathogenic Arsenophonus strains. bioRxiv. DOI: https://doi.org/10.1101/2024.08.06.606843

POSTER 28 cancelled

Unraveling multitrophic interactions of floral nectar microorganisms: their above- to belowground influence

Claudia Marina LÓPEZ-GARCÍA (1), Indira Aranza RODRÍGUEZ-GÓMEZ (1), Ken Oyama Nakagawa (1), Frédérique REVERCHON (2,3), Alfonso MÉNDEZ-BRAVO (1,3)

1 Escuela Nacional de Estudios Superiores Unidad-Morelia, Universidad Autónoma de México, Mexico
2 Red de Diversidad Biológica del Occidente Mexicano, Centro Regional del Bajío, Instituto de Ecología, A.C., Mexico
3 UMR Interactions Arbres-Microorganismes, INRAE Grand-Est Nancy, France

Floral nectar represents the most important reward for pollinators and provide a restrictive habitat for specialized yeasts and bacteria. The nectar inhabiting microbiota contributes to flower protection and can influence mutualistic relationships between plants and pollinators, ultimately affecting pollination success. Unlike most floral nectars, avocado nectar does not contain high amounts of glucose but is almost exclusively constituted by sucrose and perseitol. The influence of nectar microbes on belowground interactions, particularly with the plant root system have been scarcely studied, and if we consider that more than 99% of the flowers produced at anthesis in avocado are not able to set fruits, falling to the ground, the ability of this microbiota to interact with the roots of their hosts results as an obligated ecological phenomenon to study. Our objectives were to screen nectar culturable microorganisms for beneficial properties in the avocado-honey bees pollination system by evaluating their antagonistic activity against the avocado pathogens Phytophthora cinnamomi and Colletotrichum gloeosporioides, and against the most devastating honey bee pathogens Ascosphaera apis and Paenibacillus larvae; as well as their ability to promote plant development in Arabidopsis thaliana. From the 43 evaluated microbial morphotypes, at least 20, belonging to bacteria genera Pseudomonas, Dietzia, Nocardioides, Streptomyces, Klebsiella, Curtobacterium and Paenibacillus, and the yeast-like fungi Aureobasidium and Filobasidium showed differentially antagonistic activity against the pathogens and growth promoting properties in Arabidopsis, by inducing hormonal signaling pathways involved in development and defense responses. Collectively, our findings highlight the selectivity of avocado floral nectar over its inhabiting microorganisms, evidencing their potential beneficial effects in the avocado-honeybees pollination system and the influence on the belowground interactions.

References:

Martin et al., 2021. Phil.Trans. R. Soc. B.

Mueller et al., 2022. Environ. Microbiol.

Breaking the TALE: How Xanthomonas phaseoli pv. manihotis rewrites the script with SWEET genes

Carlos ZARATE-CHAVES (1), Francisco SANCHEZ (2), Martin DOMINGUEZ (3), Chloé COSSIN (1), Sarah DELAFOSSE (1), Zoé ROUX (1), Jeniffer YANEZ (4), Emilie THOMAS (1), Florence AUGUY (1), Alexis DEREEPER (1), Sébastien CUNNAC (1), Ralf KOEBNIK (1), Paula DIAZ-TATIS (5), Paul CHAVARRIAGA (2), Adriana BERNAL (6), Boris SZUREK (1)

1- PHIM, Univ Montpellier, IRD, CIRAD, INRAe, Institut Agro, Montpellier, France
2- Alliance Bioversity Internaonal and CIAT, Palmira, Colombia
3- Instituto Nacional deTecnología Agropecuaria, Cerro Azul, Argentina
4- Pontificia Universidad Catolica del Ecuador, Ciencias naturales y exactas, Quito, Ecuador
5- Manihot Biotec, Dpto de Biología, Universidad Nacional de Colombia, Bogotá, Colombia
6- LIMMA, Universidad de los Andes, Bogotá, Colombia

Sugars Will Eventually be Exported Transporters (SWEETs) are key susceptibility (S) factors that Xanthomonas species exploit in crops like rice [1], cotton [2], and cassava [3]. In Cassava Bacterial Blight, Xanthomonas phaseoli pv. manihotis (Xpm) uses cassava MeSWEET10a as an S gene, activating it through Transcription Activator-Like Effectors (TALEs), specifically TAL20 [3] and TAL22 [4]. So far, this is the only S gene identified for the Xpm-cassava interaction [3-5]. However, some pathogenic Xpm strains lack the MeSWEET10a-activating TALE variants. Comparative genomics and pathogenicity tests on a global collection of Xpm strains revealed that some isolates can alternatively activate MeSWEET10e, another clade-III SWEET transporter in cassava. In this study, we confirmed MeSWEET10e as an S gene and developed cassava edited lines showing increased resistance to Xpm strains that activate MeSWEET10e. We found that activation of MeSWEET10e is independent of TALEs, but relies on a type-three effector (Xop). Comparative genomics pointed to a plasmid-borne Xop with a unique architecture resembling TALEs, though its role is still being investigated. A recent survey of Xpm strains from Argentinian fields confirmed that this molecular mechanism and the S gene described here are currently used by Xpm populations to cause disease. This is the first reported case of TALE-independent SWEET activation in the Xanthomonas genus, which implies that Xops could act as key pathogenicity factors to the same extent of TALEs.

References:

1 P. K. Gupta, H. S. Balyan, T. Gautam, SWEET genes and TAL effectors for disease resistance in plants: Present status and future prospects. Mol. Plant Pathol. 22, 1014–1026 (2021).

2 K. L. Cox, et al., TAL effector driven induction of a SWEET gene confers susceptibility to bacterial blight of cotton. Nat. Commun. 8, 15588 (2017).

3 M. Cohn, et al., Xanthomonas axonopodis virulence is promoted by a Transcription Activator-Like effector-mediated induction of a SWEET sugar transporter in cassava. Mol. Plant-Microbe Interact. 27, 1186–1198 (2014).

4 C. A. Zárate-Chaves, et al., TAL effector repertoires of strains of Xanthomonas phaseoli pv. Manihotis in commercial cassava crops reveal high diversity at the country scale. Microorganisms 9, 1–26 (2021).

5 C. A. Zárate-Chaves, et al., Cassava diseases caused by Xanthomonas phaseoli pv. manihotis and Xanthomonas cassavae. Mol. Plant Pathol. 22, 1520–1537 (2021)..

Fight or cooperate? How Alnus glutinosa tells its symbiont Frankia alni from the pathogen Phytophthora alni

Mathilde Vincent (1), Aude Herrera-Belaroussi (1), Pascale Fournier (1), Nicolas Parisot (2), Nawelle Saïdi (1), Guylaine Miotello (3), Claire Prigent-Combaret (1), Jean Armengaud (3), Anne-Emmanuelle Hay (1) and Hasna Boubakri (1)

1 Université Claude Bernard Lyon 1, CNRS, UMR 5557, INRAE UMR1418, Ecologie Microbienne, F-69622, Villeurbanne, France ;

2 INSA-Lyon, INRAE, UMR203 BF2i, Biologie Fonctionnelle Insectes et Interactions, Villeurbanne, France ;

3 Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS), SPI, F-30200, Bagnols-sur-Cèze, France

Plants are constantly interacting with their surrounding environment. These interactions, whether biotic or abiotic, can be favorable or unfavorable for the plant. Among biotic interactions, some may involve symbiotic microorganisms or pathogens. Faced with the multitude of these interactions, plants have developed mechanisms for recognizing microorganisms in order to establish symbiosis, in the case of beneficial microorganisms, or to develop defense mechanisms to counter pathogens. To understand the plant immune response to a symbiont or a pathogen, we set up an experiment involving the inoculation of Alnus glutinosa with a symbiont (Frankia alni) or a pathogen (Phytophthora alni sp alni) and analyzed the molecular response of the plant after 1 and 3 days post inoculation. Our results revealed that after 1 dpi, plants activated same the biological processes against the symbiont and pathogen, notably via the induction of genetic markers putatively invovled in oxidative stress . Conversely, after 3 days of inoculation, more specific responses seem to emerge in reaction to each microbial interaction.

Finally, when the plant is co-inoculated with both microorganisms at the same time, the responses of the plant are turned towards defence against the pathogen, which prevents the establishment of symbiosis.

References:

Exploring the transmission routes of bacteria from microbiota to seed

Thomas CHADELAUD (1), Zian ACKER (1), Thomas REDOIS (1), Logan SUTEAU (1), Louna COLAERT-SENTENAC (1), Matthieu BARRET (1), Armelle DARRASSE (1)

(1) Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, F-49000 Angers, France

Seeds are a habitat for a vast array of bacteria, some of which are beneficial, commensal, and pathogenic for host plants. According to the available literature, three main routes of seed transmission have been identified for plant pathogen. These include external transmission by contact, floral transmission and internal transmission via the xylem (Maude, 1996). Nevertheless, the relative importance of each of these routes in the seed transmission of bacterial members of the microbiota is poorly understood. The objective of this work was to detail these transmission routes to bean seeds (Phaseolus vulgaris var. Flavert) for a diversity of bacterial strains representative of the populations most frequently detected within the microbiota.

The relative contribution of each route was estimated for thirteen strains from five distinct bacterial families following inoculation into bean stems, flowers and pods. As controls, we used the wild-type strain Xanthomonas citri pv. fuscans CFBP7767, a pathogenic bacterium frequently transmitted to bean seeds (Darrasse et al., 2018) and its ΔhrcV mutant, impaired in the type III secretion system. Although X. citri pv. fuscans can be transmitted via all three routes, the internal route was the most efficient. In contrast the other bacterial strains selected in this work were neither transmitted to mature seeds by the internal nor the floral routes. These initial results raise questions about the pathways used by these bacteria to colonize bean seeds. New experiments are currently in progress to assess the ability of the strains to vascularise and disseminate through the xylem.

References:

1.Darrasse, A., Barret, M., Cesbron, S., Compant, S. & Jacques, M.-A. Niches and routes of transmission of Xanthomonas citri pv. fuscans to bean seeds. Plant Soil 422, 115–128 (2018).

2.Maude, R. B. Seedborne Diseases and Their Control: Principles and Practice. (1996).

Study of the Smc-dependent genome topology of Sinorhizobium meliloti in free-living condition and symbiosis

Sara DENDENE (1), Fazal HAQ (1), Amanda EDLING DE BARROS (1), Victoria LIOY (1), Peter MERGAERT (1), Emanuele G. BIONDI (1)

(1) I2BC CNRS Gif sur Yvette

The symbiosis between the alphaproteobacterium Sinorhizobium meliloti and Medicago sativa, is a well-studied model (Jones et al., 2007). This symbiosis leads to the production of a symbiotic organ called nodule, which is colonized by nitrogen fixing bacteria. Several studies have showed that during this symbiosis process the bacteria undergo a terminal differentiation into bacteroids, characterized by elongated cells, higher permeability and an endoreduplication of the genome, which reaches up to 32 copies against 2 maximum copies in free living conditions (Mergaert et al., 2006). Since the DNA genome topology may be linked to the transcription profile, we hypothesize that the highly polyploid genome should be compacted in the nodule thus, therefore affecting the unique transcriptional program of bacteroids. We recently demonstrated by Hi-C experiments that this differentiation involves also a massive reorganization of the genome architecture, controlled in bacteria by a plethora of proteins never studied in S. meliloti, such as the Smc complex (Song and Loparo, 2015). Here, by the use of mutants of genes of the Smc complex, we studied its role in free living conditions and during the terminal differentiation. In order to do that, we carried out a combination of molecular analysis, including RNAseq and HiC. A deep understanding of the mechanism underlying the symbiosis establishment process, will allow us in the future to use strains with an enhanced symbiosis efficiency.

References:

Jones, K.M., Kobayashi, H., Davies, B.W., Taga, M.E., and Walker, G.C. (2007) How rhizobial symbionts invade plants: the Sinorhizobium-Medicago model. Nat Rev Microbiol 5: 619–633.

Mergaert, P., Uchiumi, T., Alunni, B., Evanno, G., Cheron, A., Catrice, O., et al. (2006) Eukaryotic control on bacterial cell cycle and differentiation in the Rhizobium-legume symbiosis. Proc Natl Acad Sci USA 103: 5230–5235.

Song, D., and Loparo, J.J. (2015) Building bridges within the bacterial chromosome. Trends Genet 31: 164–173.

A Community-Curated DokuWiki Resource on Diagnostics, Diversity, Pathogenicity, and Genetic Control of Xanthomonads

Joana COSTA (1,2), Joël F. POTHIER (3), Eran BOSIS (4), Jens BOCH (5), Roland KÖLLIKER (6) and Ralf KOEBNIK (7)

(1) University of Coimbra, Centre for Functional Ecology—Science for People & the Planet, Associate Laboratory TERRA, Department of Life Sciences, Coimbra, Portugal

(2) Laboratory for Phytopathology, Instituto Pedro Nunes, Coimbra, Portugal

(3) Environmental Genomics and Systems Biology Research Group, Institute of Natural Resource Sciences, Zurich University of Applied Sciences (ZHAW), Wädenswil, Switzerland

(4) Department of Biotechnology Engineering, Braude College of Engineering, Karmiel, Israel

(5) Institute of Plant Genetics, Leibniz Universität Hannover, Hannover, Germany

(6) Molecular Plant Breeding, Institute of Agricultural Sciences, ETH Zurich, Zurich, Switzerland

(7) Plant Health Institute of Montpellier, University of Montpellier, CIRAD, INRAE, Institut Agro, IRD, Montpellier, France

Xanthomonads, including Xanthomonas and Xylella species, constitute a large and significant group of economically and ecologically important plant pathogens. Up-to-date knowledge of these pathogens and their hosts is essential for the development of suitable control measures. Traditional review articles or book chapters have inherent limitations, including static content and rapid obsolescence. To address these challenges, we have developed a Web-based knowledge platform dedicated to xanthomonads, inspired by the concept of living systematic reviews (1). This platform offers a dynamic resource that encompasses bacterial virulence factors, plant resistance genes, and tools for diagnostics and genetic diversity studies. Our goal is to facilitate access for newcomers to the field, provide continuing education opportunities for students, assist plant protection services with diagnostics, provide valuable information to breeders on sources of resistance and breeding targets, and offer comprehensive expert knowledge to other stakeholders interested in plant-pathogenic xanthomonads. This resource is available for queries and updates at https://euroxanth.ipn.pt.

References:

(1) Costa J, Pothier JF, Bosis E, Boch J, Kölliker R, Koebnik R (2024). A Community-Curated DokuWiki Resource on Diagnostics, Diversity, Pathogenicity, and Genetic Control of Xanthomonads. Mol. Plant Microbe Interact. 37: 347-353. doi: 10.1094/MPMI-11-23-0184-FI

Agrobacterium extracellular vesicles : composition and roles in Agrobacterium lifestyles

Timothée ZANNIS-PEYROT (1), Isabelle KERZAON (1), Vincent GAILLARD (1), Jeanne DORE (1), Pierre-Yves DUGAS (2), Matthieu GAUCHER (4), Fabiola BASTIAN (3), Ludovic VIAL* (1), Céline LAVIRE* (1)

(1) Université Claude Bernard Lyon 1, UMR 5557 Ecologie Microbienne, CNRS, INRAE, VetAgro Sup, UCBL, F-69622, Villeurbanne, Lyon, France
(2) Université Claude Bernard Lyon 1, CNRS, CPE Lyon, UMR 5128, Laboratoire Catalyse, Polymérisation, Procédés et Matériaux (CP2M), 69616 Villeurbanne, France
(3) DTAMB, FR BioEEnVis, Université Claude Bernard Lyon 1, Lyon, France
(4) IRHS-UMR1345, Université d'Angers, INRAE, Institut Agro, SFR 4207 QuaSaV, F-49071, Beaucouzé, France

* Both authors participated equally to this work

Agrobacterium fabrum (a species of the Agrobacterium tumefaciens species complex) exhibits diverse lifestyles. It is capable of establishing commensal relationships with the plant, but is also, under specific conditions (presence of a tumor inducing plasmid (pTi) and induction of virulence via plant compounds) a phytopathogenic bacteria responsible for Crown Gall disease (1,2). Recently, our team and other studies have demonstrated that the growth conditions of phytobacteria is a key factor modulating the production and content of bacterial extracellular vesicles (BEVs). Bacterial extracellular vesicles are lipidic shuttles that facilitate the export of cellular materials over considerable distances from the cell. BEVs can transport lipids, proteins, nucleic acids, and metabolites. BEVs, through their molecular cargos, are thought to play roles in host colonization and immune response induction during plant-bacteria interactions (3, 4). In the present study, we aimed to respond to three questions considering Agrobacterium fabrum C58 BEVs : i) How does the environment (such as plant metabolites) and/or bacterial lifestyle modify the BEVs cargo? ii) How can these BEVs modulate the plant physiology? iii) Does the plant respond in the same way when exposed to either bacteria or only BEVs? A. fabrum C58 BEVs were visualized using electron microscopy and the effect of virulence conditions on BEVs cargos was characterized using LC-MS² analyses. Finally, after an exposure to the roots to either BEVs or A. fabrum C58 cells, we compared the specialized metabolite profiles of aerial parts and roots of Solanum lycopersicum. Our results indicate that virulence conditions affect the molecular content of BEVs produced by A. fabrum C58 and that A. fabrum C58 BEVs also impacts S. lycopersicum specialized metabolites profiles in a different manner than the bacterium itself.

References:

(1) Nester, E.W. (2015) Agrobacterium: nature's genetic engineer. Front Plant Sci 5:.

(2) Meyer, T., Renoud, S., Vigouroux, A., Miomandre, A., Gaillard, V., Kerzaon, I., et al. (2018) Regulation of Hydroxycinnamic Acid Degradation Drives Agrobacterium fabrum Lifestyles. Mol Plant-Microbe Interactions® 31: 814–822.

(3) Janda, M., Ludwig, C., Rybak, K., Meng, C., Stigliano, E., Botzenhardt, L., et al. (2021) Biophysical and proteomic analyses suggest functions of Pseudomonas syringae pv tomato DC3000 extracellular vesicles in bacterial growth during plant infection. 2021.02.08.430144.

(4) McMillan, H.M., Zebell, S.G., Ristaino, J.B., Dong, X., and Kuehn, M.J. (2021) Protective plant immune responses are elicited by bacterial outer membrane vesicles. Cell Rep 34: 108645.

Is Xanthomonas campestris pv. campestris an emerging pathogen on winter oilseed rape?

Sophie CESBRON, Martial BRIAND, Sophie Grandet, Agathe BRAULT, Jessica DITTMER, Alain SARNIGUET

UMR 1345, Université d'Angers, Institut Agro, INRAE, IRHS, Angers, France

Xanthomonas campestris pv. campestris (Xcc), the agent of black rot, usually significantly affects cabbage and other cruciferous vegetables. Typical v-shaped necrotic lesions caused by Xcc have also been observed in fields on winter oilseed rape (WOSR), Brassica napus L, in Serbia (Popovic et al, 2013) and more recently in western France (Cesbron et al, 2024). Therefore, we ask questions related to the possible emergence of the disease on WOSR: i) is it a dispersal of Xcc from other Brassicacea reservoirs or areas, ii) is it caused by new Xcc strains, iii) is it the consequence of selection by new WOSR hosts? We first studied the search for new characteristics by comparing the high-quality genomic sequences (using long-read technology) of a set of strains recently isolated from WOSR (n= 18) and of all the Xc strains (n=115) available on the databases. An initial phylogenetic MLST analysis based on 100 common genes revealed a grouping of WOSR strains in 2 clades that differ significantly from Xcc strains isolated from other Brassicacea. Although novel Transcription Activator-Like Effectors (TALEs) were described, the WOSR strains in these clades did not differ from other Xcc in their repertoire of type III–secreted effectors or in their plasmid content. All the WOSR isolated strains were very aggressive on all the genotypes of a collection representative of B. napus genetic diversity. Seed lots that led to outbreaks in the field were contaminated by Xcc. This observation leads to the hypothesis of seed-to-seed transmission and is now being tested. Altogether these data suggest a zonal shift, do not provide sufficient evidence for the selection of new traits but clade specificity and probable dispersal of Xcc through the global seed trade. The risk of spread of the disease to WOSR and other brassica crops in France will be discussed.

References:

Popović, T., Balaž, J., Starović, M., Trkulja, N., Ivanović, Ž., Ignjatov, M., & Jošić, D. (2013). First report of Xanthomonas campestris pv. campestris as the causal agent of black rot on oilseed rape (Brassica napus) in Serbia. Plant Disease, 97(3), 418-418.

Cesbron, S., Briand, M., Dittmer, J., Bousset-Vaslin, L., Jacques, M. A., & Sarniguet, A. (2024). First Report of Xanthomonas campestris pv. campestris Causing Black Rot on Oilseed Rape (Brassica napus) in France. Plant Disease, 108(3), 784.

Genomic insights into Xylella fastidiosa: tracking subspecies pauca ST53 in France

Amandine CUNTY (1), Déborah MERDA (2), Anne-Laure BOUTIGNY (1), Bruno LEGENDRE (1), Valérie OLIVIER (1)

(1) Anses, Plant Health Laboratory, BVO unit, Angers, France

(2) Anses, Laboratory of food safety, SPAAD unit, Anses, Maisons-Alfort, France

Xylella fastidiosa is a plant-pathogenic bacterium that is native to the Americas. It has a wide host range and causes significant diseases in economically important crops, including grapevine, citrus, and olive trees. X. fastidiosa has been identified in Europe over the past few decades, with the detection of several subspecies (multiplex, fastidiosa and pauca) and sequence types (ST) in various plant species across Italy, France, the Balearic Islands, Spain and Portugal since 2013.

Whole genome sequencing data are essential for comparative genomics, epidemiology and for determining the origin of introductions. Nevertheless, the isolation of X. fastidiosa from contaminated plant material is not always successful, which makes it impossible to obtain a complete genome sequence.

A SureSelect targeted enrichment method was developed and the enrichment process was found to be highly effective in recovering the entire genome sequence, with significantly improved genome coverage, regardless of the plant species or level of contamination (Boutigny et al., 2023).

This novel approach was applied to two distinct plant samples contaminated with the subspecies pauca (ST53), which were identified on two occasions (in 2015 and in 2019) in a limited geographical area in the PACA region in Menton (Denancé et al., 2017; Cunty et al., 2020). Phylogenetic and genomic comparisons were conducted using the two bacterial genome sequences captured and a range of X. fastidiosa subspecies pauca genomic sequences from a public database, including ST53 from Italy and Costa Rica. The results obtained from these different approaches revealed a link between the Italian and French strains. A tip-dating analysis was performed on these data, which allowed the most probable scenario of introduction of these strains into France to be inferred.

References:

Boutigny A-L, Remenant B, Legendre B, et al., 2023. Direct Xylella fastidiosa whole genome sequencing from various plant species using targeted enrichment. Journal of microbiological methods 208, 106719.

Cunty A, Legendre B, De Jerphanion P, et al., 2020. Xylella fastidiosa subspecies and sequence types detected in Philaenus spumarius and in infected plants in France share the same locations. Plant Pathology 69, 1798-811.

Denancé N, Legendre B, Briand M, et al., 2017. Several subspecies and sequence types are associated with the emergence of Xylella fastidiosa in natural settings in France. Plant Pathology 66, 1054-64.

Comparative genomics of Xylella fastidiosa subsp. multiplex strains from France: Genetic diversity and pathogen dynamics

Jessica Dittmer (1), Thinh Vo Hoang Quoc (1), Martial Briand (1), Bruno Legendre (2), Amandine Cunty (2), Anne-Laure Boutigny (2), Killian Grilloton (1), Sophie Cesbron (1), Muriel Bahut (3), Marie-Agnès Jacques (1)

1) Université d'Angers, Institut Agro, INRAE, IRHS, SFR Quasav, Angers, France

2) ANSES Plant Health Laboratory (LSV), Angers, France

3) Université d'Angers, SFR Quasav, Angers, France

Xylella fastidiosa is a plant pathogen responsible for numerous crop diseases worldwide. It specifically colonizes the xylem and is naturally transmitted exclusively by sap-feeding insects. X. fastidiosa has a significant adaptive capacity, as evidenced by its high genetic diversity and frequent recombination events between subspecies. Originating from the Americas, X. fastidiosa is now present in several European countries due to accidental introductions of infected plant material. This situation calls for a better understanding of the evolutionary dynamics of the pathogen in its new areas of distribution. Herein, we present a comparative genomics analysis of 75 strains belonging to the subspecies multiplex associated with various host plants in France since the first detection of the pathogen in 2015. High-quality genome sequences were obtained using both PacBio and Oxford Nanopore sequencing technologies. Phylogenomics and comparative genomics analyses were used to determine the phylogenetic position of the French strains within the subspecies multiplex and to analyse differences in functional gene content within and between different sequence types (ST) present in France. Our results revealed that ST6 strains are more genetically diverse than ST7 strains, along with regional differences in gene content between ST6 strains from Occitanie compared to ST6 strains from Corsica and PACA. This suggests multiple independent introductions of ST6 strains to France and different evolutionary dynamics between ST6 and ST7. In addition, we identified numerous ST6-specific genes involved in microbial interactions with bacteria and phages and several strains harboured a conjugative plasmid, in contrast to American strains of this subspecies which rarely possess plasmids. We will discuss the putative functions and acquisition routes of these genes and plasmids as well as their potential role in the adaptation to new host plants after pathogen introduction.

References:

Diversity of Xanthomonas species isolated on Juglans regia L. in its cradle in Central Asia

Marion Fischer-Le Saux (1), Géraldine Taghouti (1), Tatiana Kostritsyna (2), Timur Turdiev(3), Logan Suteau (1), Véronique Decroocq (4)

(1) Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, F-49000 Angers, France

(2) International Higher School of Medicine, Bishkek, Kyrgyzstan

(3) Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan

(4) Univ. Bordeaux, INRAE, UMR 1332 Biologie du Fruit et Pathologie, Villenave d'Ornon, France

Xanthomonas arboricola pv. juglandis (Xaj) is the causal agent of walnut blight, a disease in continuous expansion and with major economic impacts on walnut production. Xaj represent an epidemic clone that was first described in the USA at the beginning of the twentieth century. It is phylogenetically related to pathovar pruni and pathovar corylina which attack Prunus spp. and hazelnut respectively. It has been shown that the centre of origin of cultivated crops could play an important role in pathogen emergence. Juglans regia L. and Prunus share the same cradle in Central Asia. The concomitant presence of Juglans regia L. and wild apricot trees in the forests of the Tian-Shan mountains in Central Asia has reinforced our concern about the role of Xanthomonas populations from the centre of origin in the evolutionary history of these pathogens. A survey was conducted in 2019 in Kazakhstan and kyrgyzstan to get preliminary data about the presence of Xanthomonas in wild walnut and Prunus populations. No Xanthomonas strains were isolated from Prunus trees despite the presence of typical shot hole symptoms. Fifteen strains of Xanthomonas were isolated from walnut trees showing typical symptoms of walnut blight. Isolates were further identified by multilocus sequence analysis. No Xaj strains were identified from wild population of Juglans regia L. The few Xanthomonas strains isolated from natural populations of walnut trees belonged respectively to X. hortorum, X. euroxantheae and a genomospecies in the vicinity of X. arboricola. The isolates were further characterized by genome sequencing and phenotypic tests.

References:

The peculiar cell wall polysaccharides of plant hydathodes are remodelled following *Xanthomonas* plant vascular pathogen infection

Jean-Marc Routaboul[1], Caroline Bellenot[1], Alain Jauneau[2], Marie-Françoise Jardinaud[1], Sébastien Carrère[1], Marie-Christine Auriac[1,2], Marielle Aguilar[3], Aude Cerutti[1], Emmanuelle Lauber[1], Vincent Burlat[3], Laurent D. Noël[1]

[1] LIPME, Université de Toulouse, INRA, CNRS, F-31326 Castanet-Tolosan.

[2] Plateforme Imagerie, Institut Fédératif de Recherche 3450, Pôle de Biotechnologie Végétale, F-31326 Castanet-Tolosan.

[3] Laboratoire de Recherche en Sciences Végétales, LRSV, CNRS, UPS, Université de Toulouse, Castanet-Tolosan, France

Xanthomonas campestris pv. *campestris* (*Xcc*) bacteria, the causal agent of black rot disease on brassica crops (such as cauliflower), belong to a growing number of vascular pathogens entering leaf through hydathodes. Hydathodes are organs found at the leaf margins of all vascular plants which release xylem-derived droplets to avoid detrimental leaf water flooding. Below the hydathode pores lies a loosely connected tissue soaked in guttation fluid that offers a favourable environment for microbial growth (Routaboul *et al.,* 2024; Bellenot *et al.,* 2022) and a direct connection to the xylem vessels (Cerutti *et al.,* 2017). To study the plant immune responses that take place in hydathode during the early stages of infection, we studied the transcriptome of cauliflower hydathodes infected with a virulent or an avirulent *Xcc* strain. We found that the basal immune response at one day post infection (dpi) was similar in both *Xcc* strains. At 3dpi, the number of differentially expressed genes (DEGs) was lower compared to 1dpi but still 415 DEGs (FDR<0.001) were detected comparing the virulent to the avirulent *Xcc* strain. Those include some plant cell wall modification (Pectin Methyl Esterases and inhibitors) and degradation (Pectin Lyases) related genes that are also differentially expressed in hydathodes (Routaboul *et al.,* 2024). Immunofluorescence labelling of cell wall polysaccharides in healthy and infected hydathodes showed that hydathode contains partially methyl-esterified pectins that are the first cell wall component degraded following *Xcc* infection. These transcriptomic and topochemical atlas provide molecular cues to hydathode identity and specificities under attack by a pathogen.

References:

Cerutti *et al.,* (2017) *Plant Physiol.* 174: 700–716.

Bellenot *et al.,* 2022 Current Biol. 32: R763–R764

Routaboul *et al.,* (2024) *Plant J.* in press.

Optimizing Silicon Availability in Agriculture: Enhancing Plant Growth Through Silicon-Solubilizing Microbes and Prebiotic Technology

Quentin DROGUE (1) ; Laurie DUTRESSOULE (1) ; Mustapha ARKOUN (1) ; Sylvain PLUCHON (1)

1 Agro Innovation International, Saint Malo, France.

Among the various essential nutrients in an agricultural context, silicon holds a special place by playing several important roles. This element, considered beneficial, notably helps to enhance plant resistance to environmental stresses, improve their growth, and promote the efficient absorption of nutrients by the plant. Although silica is very abundant in the soil, it is primarily found in mineral and organic forms and is therefore not directly available to plants. Thus, to make it bioavailable and enable its absorption at the root level, it is essential to promote the activity of some microorganisms present in the soil called Silicon Solubilizing Microbes (SSM). The objective of this work was: i) to isolate and quantify the proportion of the SSM pool from agricultural soil samples taken from the field; ii) to test and develop a technological solution with prebiotic properties specifically aimed at promoting SSM activity; and iii) to validate the effectiveness of this technology on yield in the field. After sampling, isolation, purification, and sequencing, the initial results confirm the presence of SSM and a moderate microbial diversity in fields with conventional fertilization and agricultural practices. The prebiotic action of a technological agent promoting SSMs was evaluated in the laboratory on reference SSM strains. The results showed a significant increase (up to +53% in vitro) in the growth of these strains and their ability to solubilize silica. Finally, field trials conducted on a barley crop confirmed the effectiveness of this technology on plant growth and yield, resulting in an increase of +8% and a gain of +5 quintals compared to the control.

This study enabled the evaluation and development of a technical solution of interest for agriculture, which promotes, through a prebiotic effect, the existing SSM pool in the soil, thus enhancing the beneficial effect of silicon for plants.

References:

- Naureen, Z., Aqeel, M., Hassan, M.N., Gilani, S.A., Bouqellah, N., Mabood, F., Hussain, J. and Hafeez, F.Y. (2015) Isolation and Screening of Silicate Bacteria from Various Habitats for Biological Control of Phytopathogenic Fungi. American Journal of Plant Sciences, 6, 2850-2859. http://dx.doi.org/10.4236/ajps.2015.618282

- Mahbod Sahebi, Mohamed M. Hanafi, Abdullah Siti Nor Akmar, Mohd Y. Rafii,

Parisa Azizi, F. F. Tengoua, Jamaludin Nurul Mayzaitul Azwa, and M. Shabanimofrad, (2014) Importance of Silicon and Mechanisms of Biosilica Formation in Plants. BioMed Research International. http://dx.doi.org/10.1155/2015/396010

- N. Vasanthi, Lilly M. Saleena and S. Anthoni Raj, (2014) SILICON IN CROP PRODUCTION AND CROP PROTECTION - A REVIEW. Agri. Reviews, 35 (1) : 14-23, 2014. DOI- 10.5958/j.0976-0741.35.1.002

POSTER 42 cancelled

Identification of type III effectors in Xylophilus ampelinus may inform integrated managment the Oléron's disease of grapevine

Zoe ROUX (1), Naama WAGNER (2), Tal PUPKO (2) and Ralf KOEBNIK (1)

(1) Plant Health Institute of Montpellier, University of Montpellier, CIRAD, INRAE, Institut Agro, IRD, Montpellier, France

(2) Shmunis School of Biomedicine and Cancer Research, Tel Aviv University, Israel

Bacterial blight of grapevine, also known as "maladie d'Oléron" in France, is caused by the slow-growing bacterium Xylophilus ampelinus (Willems et al., 1987), formerly known as Bacillus vitivorus (Baccarini, 1893), Erwinia vitivora (Du Plessis, 1940) and Xanthomonas ampelina (Panagopoulos, 1969). Symptoms include discoloration of young shoots, necrotic spots on leaves, reddish-brown streaks on shoots, cracks, cankers, wilting and dieback (1). Disease severity depends on cultivar susceptibility and environmental conditions (2). The bacterium is reported as vascular pathogen colonising the xylem, forming biofilms. Later, also phloem and cambial tissues were found to be infected (3). To shed light into the biology of this pathogen, we scrutinized all available genomes of the genus for the presence of type III secretion systems (4), predicted type III effectors using a machine-learning technique (5) and confirmed two of them to contain a funtional type III secretion signal using an AvrBs1 reporter approach (6). The presence of type III effectors suggests that effector-triggered immunity may exist in grapevine or non-host plants and that strategies targeting type III effectors for resistance engineering may contribute to suitable control measures.

References:

(1) EFSA Panel on Plant Health (2014). Scientific Opinion on the pest categorisation of Xylophilus ampelinus (Panagopoulos) Willems et al. EFSA Journal 12: 3921.

(2) Peros JP, Berger G, Ride M (1995) Effect of grapevine cultivar, strain of Xylophilius ampelinus and culture medium on in vitro development of bacterial necrosis. Vitis 34: 189-190.

(3) EPPO (2009). Xylophilus ampelinus . Bulletin OEPP/EPPO Bulletin 39: 403-412.

(4) Portier P, Taghouti G, Bertrand PE, Briand M, Dutrieux C, Lathus A, Fischer-Le Saux M (2022). Analysis of the diversity of Xylophilus ampelinus strains held in CIRM-CFBP reveals a strongly homogenous species. Microorganisms 10: 1531.

(5) Wagner N, Avram O, Gold-Binshtok D, Zerah B, Teper D, Pupko T (2022). Effectidor: an automated machine-learning-based web server for the prediction of type-III secretion system effectors. Bioinformatics 38: 2341-2343.

(6) Zhao S, Mo WL, Wu F, Tang W, Tang JL, Szurek B, Verdier V, Koebnik R, Feng JX (2013). Identification of non-TAL effectors in Xanthomonas oryzae pv. oryzae Chinese strain 13751 and analysis of their role in the bacterial virulence. World J. Microbiol. Biotechnol. 29: 733-744.

The Plant Coumarin Scopoletin Modulates Natural Product Biosynthesis by Endophytic Streptomyces

Emtinan DIAB (1), Chao DU, Wendy TIGANI, Somayah S. ELSAYED, Gilles P. VAN WEZEL

(1) Leiden University, Netherlands

Microorganisms living in symbiosis with plants can provide beneficial functions to their host via production of natural products (NP) including biocontrol activities. However, regulation of microbial NP biosynthesis in response to plant signals is understudied. Coumarins are a well-described class of plant metabolites. These compounds play multiple roles to support plant fitness including iron solubilization and antimicrobial activity against phytopathogens (1). Several studies describe the roles of coumarins as agents controlling microbiome recruitment (2,3); however, the roles of coumarins as signals perceived by plant-associated microbes remains elusive. We sought to investigate the effect of the abundant coumarin scopoletin on NP biosynthesis by endophytic Streptomycetes, a genus of Actinobacteria renowned for its biosynthetic talents. We discovered that scopoletin alters the production of bioactive compounds by Streptomyces sp. ATMOS53 isolated from Arabidopsis roots. Using multi-omics approaches combining metabolomics, proteomics, and genomics, we showed that scopoletin interferes with the biosynthesis of anthracyclines, which are cytotoxic compounds produced by type II polyketide synthases. Scopoletin interrupts cyclization steps of the polyketide backbone, resulting in the accumulation of the shunt products UWM7 and decreased overall production of the final products of the pathway. Conversely, scopoletin stimulated the production of bohemamines, a class of bacterial pyrrolizidine alkaloids synthesized by non-ribosomal peptide synthases. This was correlated to enhanced levels of the biosynthetic enzymes. The effect of scopoletin treatment resulted in a lowered toxicity of ATMOS53 crude extract against plant associated Bacillus species. Taken together, our results highlights the importance of accounting the ecological chemistry of plant-microbe interaction studies to fully unravel the capabilities of microbes considered to be used as bio-control agents.

References:

1) Stringlis et al., 2019

2) Voges et al., 2019

3) Harbort et al, 2020

Accessing the Duckweed microbiome: Comparison of DNA extraction and 16S rRNA gene amplification methods to sequence the Duckweed-associated microbiome

Francois PRUDOT D'AVIGNY(1,2), Lusine GHAZARYAN(2), Romain BARNARD(1) and Osnat GILLOR(2)

1 Agroécologie, INRAE, Institut Agro, Univ. Bourgogne, Univ. Bourgogne Franche-Comté, 21000, Dijon, France

2 Zuckerberg Institute for Water Research, Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, 8499000, Israel

The duckweed microbiome plays a central role in several key processes, including bioremediation, nitrogen fixation, and essential biomolecule production. Despite its importance, the composition and dynamics of the duckweed microbiome remains poorly understood. The most common method to address these microbial communities is amplicon sequencing of the bacterial 16S ribosomal RNA (rRNA) gene.

However, methodological choices can affect the accuracy of microbial community assessment, the most prominent ones being i) the DNA extraction method, ii) the targeted hypervariable region of 16S rRNA gene, and iii) the primers used for amplification. Moreover, one of the biggest challenges is obtaining high-quality bacterial DNA from plant matrices that are predominantly composed of chloroplast and mitochondrial DNA.

This study aimed to evaluate the impact of these factors on DNA extraction and amplification to achieve the most accurate representation of the duckweed microbiome. Focusing on the model duckweed Spirodela polyrhiza, we compared three DNA extraction methods: two commercially available kits and the standard phenol-chloroform extraction. We tested DNA amplification both with and without blocking agents (PNA and LNA) using four different primer sets targeting various regions of the 16S rRNA encoding gene (V3-V4, V4, and V5-V6).

Our results showed that the most accurate assessment of the duckweed microbiome was obtained using one of the commercially available kit, targeting the V3-V4 region with a primer set that excluded the chloroplast 16S rRNA. Under these optimized conditions, the use of blocking agents was unnecessary, significantly reducing the cost and complexity of the analysis. Further work is currently being conducted to generalize this approach to other Duckweed species, and potentially other water plant species, for assessing their phylosphere.

References:

Specific quantification of Pantoea agglomerans: a key bacterial species of the seed microbiota

Sophie BONNEAU, Martial BRIAND, Matthieu BARRET

IRHS-UMR 1345, Université d'Angers, INRAE, Institut Agro, SFR 4207 QuaSaV, 49071, Beaucouzé, France

Plant microbiota modulates on host health and growth. Community profiling approaches revealed that Pantoea agglomerans is the most frequent and abundant bacterial species associated with seeds. We developed an absolute and targeted qPCR tool for quantification of P. agglomerans as an alternative of metabarcoding based on sequencing of gyrB. An analysis of the genomic diversity of this species has identified specific k-mers of P. agglomerans, which have been used to define a set of primers. The specificity of the primers has been initially checked in silico and further validated with strains representatives of the main bacterial populations associated with seeds. This qPCR assay allowed quantification of P. agglomerans on seeds, germinating seeds and seedlings of bean and radish. Furthermore, the quantification of this bacterial species can be normalized by the size of the total bacterial community using the qPCR primer pair Com1-769R (Samart Dorn-In, 2015) that target a portion of 16S rRNA gene.

References:

The seedling, an interesting model for studying primary succession

Matthieu BARRET, Sophie BONNEAU, Martial BRIAND, Boris COLLET, Loic RAJJOU

IRHS-UMR 1345, Université d'Angers, INRAE, Institut Agro, SFR 4207 QuaSaV, 49071, Beaucouzé, France.

Seed germination and seedling emergence are two key stages in the assembly of plant microbiota. During these early stages of plant development, many soil- and seed-borne taxa compete for seedling colonisation. To date, the functional traits associated with seedling primo-colonist are still unknown. In the course of this work we monitored the dynamics of bacterial communities during germination and emergence in two distinct plant species, bean and radish. We showed that the bacterial taxonomic and functional profiles were distinct between the aerial and root parts of young seedlings, as early as 3 days after germination. Non-targeted metabolomics approaches have identified metabolites specific to these two compartments. The links between these plant metabolites and the predicted bacterial functions will be discussed.

References:

Bacterial determinants involved in seedling colonization

Anaelle LE SIOU (1), Rémi PEYRAUD (2), Matthieu BARRET (1)

(1) IRHS-UMR 1345, Université d'Angers, INRAE, Institut Agro, SFR 4207 QuaSaV, 49071, Beaucouzé, France

(2) iMean, Toulouse, France

Seeds are a dispersal vector of many plant-associated micro-organisms. Although seeds can be considered as the starting point for the assembly of the plant microbiota, the percentage of micro-organisms transmitted to seedlings varies from 2% to 50%. These variations of transmission rate are mainly explained by competition with soil-borne taxa. This competition is mostly driven by exudation of numerous molecules during seed imbibition. My thesis project aims to explore the bacterial genetic determinants involved in successful seedling colonization. More specifically, I will address the following research questions: Q1: What is the nature of the bacterial traits involved in primo-colonisation of seedlings? Are these traits mainly associated with primary metabolism (exploitative competition) or with specialized metabolism (interference competition)? Q2: Are these traits modulated by the biotic context in which the targeted micro-organism evolves? In other words, do changes in (i) the host genotype or (ii) the initial composition of the microbial communities in the seeds modify the nature of the bacterial genetic determinants? The experimental strategy for answering these two questions will be detailed in this poster.

References:

Rochefort, A., Simonin, M., Marais, C., Guillerm-Erckelboudt, A.-Y., Barret, M., and Sarniguet, A. (2021) Transmission of Seed and Soil Microbiota to Seedling. mSystems 6: e0044621. Rochefort, A., Briand, M., Marais, C., Wagner, M.-H., Laperche, A., Vallée, P., et al. (2019)

Influence of Environment and Host Plant Genotype on the Structure and Diversity of the

Brassica napus Seed Microbiota. Phytobiomes Journal 3: 326–336. Chesneau, G., Laroche, B., Préveaux, A., Marais, C., Briand, M., Marolleau, B., et al. (2022)

Single Seed Microbiota: Assembly and Transmission from Parent Plant to Seedling. mBio 13:

e01648-22.

Preservation of Seed Microbiota : a challenge to maintain viability and function

Céline MIRGUET(1), Isa HOLLOPP(1), Clara MAIRE(1), Géraldine TAGHOUTI(1), Cécile DUTRIEUX(1), Perrine PORTIER(1)

(1)Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, CIRM-CFBP, F-49000 Angers, France

Plant-associated microbiota are complex heterogeneous communities, including non-cultivable strains. Preservation of heterogeneous community will lead to heterogenous survival, altering the taxonomic composition which in turn can alter the functionalities of the microbiota.

The preservation processes mastered by Biological Resource Centers (BRC), like deep freezing at -80°C or at -196°C in liquid nitrogen, or lyophilisation, are well adapted to isolated strains and we lack insights to understand what happens to microbiota during preservation. To overcome these limitations, the MICORBE project (https://www.microbeproject.eu/) aims at developping protocols and a framework permitting BRCs to preserve viable microbiota, in order to have these available for the future.

At CIRM-CFBP, the French Collection for Plant-associated Bacteria (https://cirm-cfbp.fr), as part of the MICROBE project, we focus on the use-case seed.

What methodology will be the most efficient and suitable for preserving the micro-organisms of seed microbiota, over the long term? All this, of course, while minimising the effects of preservation and guaranteeing viability, metabolic functions and the less altered possible taxonomic composition after preservation.

We chose to assess the taxonomic composition using metabarcoding, the metabolic profile with Biolog Ecoplates and the cultivable bacterial and fungal fraction independently of each other. All of this before and after 9 different preservation conditions in liquid nitrogen.

The first results are encouraging showing that it’s possible to preserve a complex community. More analyses are necessary to finely determine how the microbiota are altered during preservation. These experiments will enable us to refine the process to ultimately propose the best suited option for long term plant-microbiota preservation, from seeds or other plant parts.

References:

Exploiting Plant-Microbiomes Networks and Synthetic Communities to improve Crops Fitness

Caroline DE TENDER (1), Ralf KOEBNIK(2), Ahmed ABDELFATTAH (3), Gabriele BERG (4), Elodie VANDELLE (5), Gordana RACIC (6), Joël F. POTHIER (7), Renata ARTIMOVA (8), Anna BONATERRA (9), Claire PRIGENT-COMBARET (10), Francesco SPINELLI (11), Conceiçáo SANTOS (12)

(1) Ghent University, Belgium, (2) IRD Montpellier, France, (3) Leibniz Institute for Agricultural Engineering and Bioeconomy, Germany, (4) Graz Universiy of Technology, Austria, (5) University of Verona, Italy, (6) Educons University, Serbia, (7) Zürich University of Applied Sciences, Switzerland, (8) Institute of Forest Ecology, Slovakia, (9) University of Girona, Spain, (9) University Lyon, France, (10) University of Bologna, Italy, (11) University of Porto, Portugal

Plants and their microbiome will be exposed to several stress factors in the coming years, either abiotic such as drought and heat, and/or biotic including new plant pathogens. Current agricultural practices in Europe depend on agrochemicals and large amounts of water and will therefore not be sufficient to cope with the coming stresses. The COST Action MiCropBiomes (CA22158) builds on the urgent need for a transition to sustainable agriculture to ensure food security and safety by coordinating research and developing knowledge on crop microbiomes (and holobiomes) for application in sustainable precision agriculture. First, we will compile the available knowledge and datasets on microbiome assemblies and will decipher the phytobiome functional and molecular signaling through metagenomics techniques. Second, the Action will evaluate the available knowledge and datasets on the microbiome dynamics and the relation under specific environments with a focus on abiotic stress (drought and/or heat) and growing media (soil versus soilless systems). Third, we aim to understand the ecological processes that govern plant microbiome assembly and functions and how these are disrupted by pathogens. Finally, the Action will explore the plant microbiome as a source of beneficial associations of microorganisms, in which we make the transition from current mirobial inoculants to synthetic communities in relation to resilience against biotic and abiotic stresses in agriculture.

References:

Impact of microbial interactions in the adaptation and evolution of the phytopathogenic bacterium Xylella fastidiosa

Chloé ROUX (1),Garion FOUCHER (1), Sophie CESBRON (1), Marie-Agnès JACQUES (1), Jessica DITTMER (1)

(1) Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, F-49000 Angers, France

E-mail : jessica.dittmer@inrae.fr , chloe.roux@inrae.fr

Xylella fastidiosa (Xf) is a phytopathogenic bacterium native to the Americas, which was first detected in natural settings in Europe in 2013 [1]. This bacterium is transmitted exclusively by hemipteran insect vectors (biting-sucking), notably Cercopidae and Cicadellidae [2]. Currently, around 700 plant species are reported to host Xf [3], and this list includes plants of major socio-economic interest such as olive trees, citrus or grapevine, for which important losses have been observed [4]. Due to its direct and indirect economic impact on the plant sector and threat it represents for the environment, it is currently a priority quarantine pest in Europe. Xf can be found in Italy, Spain, Portugal, and in France since 2015 [1,5,6].

At present, very little is known about the interactions between Xf and other microorganisms (bacteria, fungi) colonizing the same niches (i.e. the plant xylem and insect foregut). Therefore, investigating the impact of microbial interactions on the adaptation and evolution of Xf is an important research avenue in order to better control Xf diseases. It is within this framework that the interactions between the phytopathogenic bacterium Xf and the microbiomes present in its two habitats are studied in this project: the plant xylem and the foregut of its insect vector. The main objectives of this study are to examine whether infection with Xf induces a restructuring of the microbiome colonizing the same habitats (dysbiosis), whether the different subspecies and genotypes of Xf differ in their interactions with the host-associated microbiota and to investigate the potential impact of the xylem microbiota on the success of Xf infection.

Keywords : Xylella fastidiosa, interaction, microbiome, xylem, insect

References:

1. Saponari M, Loconsole G, Cornara D, Yokomi RK, De Stradis A, Boscia D, et al. Infectivity and Transmission of Xylella fastidiosa by Philaenus spumarius (Hemiptera: Aphrophoridae) in Apulia, Italy. J Econ Entomol. 2014;107:1316–9.

2. Chatterjee S, Almeida RPP, Lindow S. Living in two worlds: The plant and insect lifestyles of Xylella fastidiosa. Annu Rev Phytopathol. 2008;46:243–71.

3. Authority (EFSA) EFS, Cavalieri V, Fasanelli E, Gibin D, Gutierrez Linares A, La Notte P, et al. Update of the Xylella spp. host plant database – Systematic literature search up to 31 December 2023. EFSA Journal. 2024;22:e8898.

4. Janse JD, Obradovic A. Xylella Fastidiosa: Its biology, diagnosis, control and risks. Plant Pathol J. 2010;92:S35–48.

5. Olmo D, Nieto A, Adrover F, Urbano A, Beidas O, Juan A, et al. First Detection of Xylella fastidiosa infecting cherry (Prunus avium) and Polygala myrtifolia plants, in Mallorca Island, Spain. Plant Dis. 2017;101:1820–1820.

6. EPPO. EPPO Global Database [Internet]. 2015 [cited 2024 Sep 5]. Available from: https://gd.eppo.int/reporting/article-5107

Dynamic and regulation of the T6SS in the seed-associated bacterium Stenotrophomonas rhizophila

Boris TAILLEFER, Yassine DAIRY, Chrystelle BRIN, Agathe BRAULT, Alain SARNIGUET

Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, F-49000 Angers, France

Stenotrophomonas rhizophila is a seed-associated bacterium that uses its metabolic and protective abilities to support seed germination and seedling growth. S. rhizophila notably uses the type 6 secretion system (T6SS) to compete with other seed microbiota members by injecting deadly toxins (1). The T6SS is a contractile machinery that delivers toxic effectors (T6E) direclty to a target strain. S. rhizophila T6SS carries 9 putative T6E and several immunities that suppose a broad range activity and a great resistance to other T6SSs (2). In our study, we questioned the regulation and the role of each T6E in the T6SS use against seed-associated bacteria. Using fluorescence microscopy, we were able to observe the unique, polar, short and highly dynamic T6SS-4 of S. rhizophila. At the populational level, the T6SS is homogeneously expressed with always 17% of cells simultaneously firing and up to 70% of cells that has fired over 5 min, underlying its highly efficient use. We identified by RT-PCR that each of the operons of the T6SS genetic cluster is controlled by its own promoter. Among all T6Es, 6 are co-regulated with the T6SS core components and 3 are inactive or expressed in unknown conditions. Simple deletion mutants of T6E showed that not a single effector but the coupled action of two amidases Tde2 and Tde5 is responsible for the major antibacterial effect of the T6SS. We also observed that the T6SS-mediated toxicity is different depending on the target strain. Specifically, some of the T6Es are target-specific such as PAAR1-Tde DNase more effective on another Stenotrophomonas strain, or the two amidases necessary to overpass an Oxalobacteracea strain resistance. This study is the first description of the T6SS dynamic and regulation in S. rhizophila and illustrates how powerful the T6SS is to shape the plant microbiota notably through its diversified T6E repertoire.

References:

1. Tiffany Garin, Chrystelle Brin, Anne Préveaux, Agathe Brault, Martial Briand, Marie Simonin, Matthieu Barret, Laure Journet, Alain Sarniguet. The type VI secretion system of Stenotrophomonas rhizophila CFBP13503 limits the transmission of Xanthomonas campestris pv. campestris 8004 from radish seeds to seedlings. Mol. Plant. Patho. (2023). Doi:10.1111/mpp.13412

2. Tiffany Garin, Agathe Brault, Coralie Marais, Martial Briand, Anne Préveaux, Marie Simonin, Matthieu Barret, Alain Sarniguet. T6SS-mediated competition by Stenotrophomonas rhizophila shapes seed-borne bacterial communities and seed-to-seedling transmission dynamics. BioRxiv (2024). Doi:10.1101/2024.07.22.604635

CIRM-CFBP: strategic resources for plant health

Perrine PORTIER(1), Géraldine TAGHOUTI(1), Cécile DUTRIEUX(1), Marion FISCHER-LE SAUX(1)

(1) Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, CIRM-CFBP, F-49000 Angers, France.

perrine.portier@inrae.fr

Biological resource centers are repository were scientists can deposit their resources and make them available for future research. These often proved to be invaluable for plant health. Thus, the collections are the memory of our past and key for future research (Broders et al., 2022).

CIRM-CFBP, the French collection for Plant-associated Bacteria holds biological resources strategic for plant health. For now 50 years, the collection helps scientists to conduct their researches permitting them access to reliable and available resources, permitting the realisation of many research projects. Over time the type of deposited resources has evolved and shifted according to the needs and projects of the plant-pathologists community. The competences and goals of the collection have evolved too, to better fit the user's needs.

However, many gaps still exist and we observe a decrease in the deposits over time. A gap in the records may hamper identification and response to a pathogen and can have important consequences for disease management or trade.

Deposit resources in a collection have a lot of personal and community benefits. This results in sharing the necessary efforts, facilities, competences and expertise, permitting to enhance the overall quality of the preservation and to invest for the future.

CIRM-CFBP has designed an on-line questionnaire: https://tinyurl.com/yc6ww38d

The researchers interested in the plant health field are invited to share theyr inputs.

References:

Broders et al., 2022. Building More Resilient Culture Collections: A Call for Increased Deposits of Plant-Associated Bacteria. Microorganisms 2022 Vol. 10 Issue 4 Pages 741

Specific bacterial interactions trigger the production of a lipopeptide siderophore in Pseudomonas fluorescens CFBP13502

Alba NOEL (1), Dimitri BREARD (2), Guillaume CHESNEAU (1), Lauren LUI (3), Alice BOULANGER (4), Martial BRIAND (1), Sophie BONNEAU (1), Morgan PRICE (3), Alain SARNIGUET (1), David GUILET (2), Adam ARKIN (3), Matthieu BARRET (1)

(1) Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, F-49000 Angers, France

(2) Substances d'Origine Naturelle et Analogues Structuraux, SONAS, SFR4207 QUASAV, UNIV Angers, Université Bretagne Loire, 49070 Beaucouzé, France.

(3) Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, Berkeley, CA, United States of America.

(4) Laboratoire des Interactions Plantes Micro-organismes (LIPM), UMR CNRS-INRA 2594/441, F-31326 Castanet-Tolosan, France

Seed is the vector of dispersion of many micro-organisms including a variety of plant pathogens such as Xanthomonas campestris pv. campestris (Xcc) and Alternaria brassicicola on a range of Brassicaceae. Seed transmission of plant pathogen can result in disease emergence in new geographical areas. No efficient treatment is currently available for limiting the incidence of bacterial plant pathogen in seeds. Some seed-borne nonpathogenic isolates can however compete for resources and space in the seed habitat. A screening of seed-borne bacterial strains possessing antibacterial activity against the phytopathogen Xcc identified one Pseudomonas fluorescens strain (CFBP13502). Increase in generation time of Xcc was specifically induced during co-culture of Xcc with CFBP13502. A biosynthetic gene cluster induced during Xcc-CFBP13502 co-culture was identified through RNAseq. According to AntiSMASH analysis, this biosynthetic gene cluster is a non-ribosomal peptide synthase potentially involved in synthesis of a lipopeptide siderophore. Reverse-genetics approach performed on a gene encoding an acetyltransferase resulted in abolition of the antibacterial activity of the cell-free supernatant of CFBP13502. Isolation and structural identification of the metabolite produced in this interaction revealed a new lipopeptide siderophore with hydroxamate binding units.

References:

A comprehensive approach to investigate the function of the LuxR solos PsaR3 in Pseudomonas syringae pv. actinidiae

Alice REGAIOLO (1,2) *, Ilaria LUCATO (1) *, Davide DANZI (1), Ralf HEERMANN (2), Annalisa POLVERARI (1), Elodie VANDELLE (1)

(1) Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134 Verona, Italy

(2) Institute of Molecular Physiology (imP), Johannes-Gutenberg-University Mainz, Biocenter II, Microbiology and Biotechnology, Hanns-Dieter-Hüsch-Weg 17, 55128 Mainz, Germany; 3 Institute for Biotechnology and Drug Research gGmbH (IBWF), Hanns-Dieter-Hüsch-Weg 17, 55128 Mainz, Germany.

*equal contribution

Pseudomonas syringae pv. actinidiae (Psa) is the eziological agent of the bacterial canker of kiwifruit (Actinidia spp.). The most aggressive biovar Psa3 is characterized by the presence of a plasmid containing a gene encoding for a LuxR solo protein, PsaR3. Although a role of PsaR3 in Psa virulence has been proposed, the autoinducer signal(s) and the targets are still unknown.

To elucidate the function(s) of PsaR3, we conducted a transcriptomic analysis of Psa overexpressing PsaR3 in an inducible manner, which revealed the upregulation of genes associated with the type III secretion system, flagellum-related motility, together with a plasmid-borne gene cluster including psaR3 itself. Interestingly, the intergenic region (IR) separating the two operons of the cluster is a functional promoter positively regulated by PsaR3, as demonstrated with a reporter system. Since no inducer molecule was included in our experimental medium, it suggested that i) the signal(s) may be produced by Psa or, alternatively, ii) the overexpression may lead to an autoactivation of the regulator. To allow a «controlled» post-translational activation of PsaR3, we thus designed and constitutively expressed a chimera protein consisting of the DNA-binding domain of PsaR3 and the autoinducer-binding domain of the LuxR protein CviR of Chromobacterium violaceum that is activated by known AHLs. Preliminary data indicate that the chimera is not responsive to AHLs, thus supporting the hypothesis of sensor auto-activation. Moreover, first results suggest that the constitutive autoactivation may lead to a negative feedback on PsaR3 function, likely to avoid a constant virulence induction.

References:

Next-generation seed diagnostics: reliable SE-qPCR for rapid pathogen detection in treated seeds

Justine Foucher (1), Mylène Ruh (1), Isabelle Sérandat (1), Nicolas Denancé (1), Hubert Lybeert (2), Thomas Baldwin (3), Sabine Tourneur (4), Marion Cordier-Demissy (5), Louis Sorieul (6), Amandine Lê Van (1), Jaiana Malabarba (1)

(1) GEVES, Groupe d'Étude et de contrôle des Variétés Et des Semences, 25 rue Georges Morel, 49071 Beaucouzé, France.
(2) HM Clause, 83 rue Louis Saillant, 26802 Portes-lès-Valence, France.
(3) Sakata Vegetables Europe, 95 route de Pouillé, 49130 Les Ponts-de-Cé, France.
(4) Vilmorin-Mikado, route du Manoir, 49250 La Ménitré, France.
(5) GAUTIER Semences, 13630 Eyragues, France.
(6) Bayer Crop Science, Bergschenhoek, Hollande Méridionale, Pays-Bas

Pathogens transmitted through seeds can cause significant damage to crops and result in major economic losses. In tomatoes (Solanum lycopersicum) and peppers (Capsicum annuum), several bacteria are damaging such as Clavibacter michiganensis (Cm), which affects tomatoes, and Xanthomonas species (X. spp.: X. euvesicatoria, X. gardneri, X. perforans, X. vesicatoria), which impact both crops. Controlling the quality of seeds and detecting these european regulated pathogens is essential to limit their spread across regions.

The current French official method for detecting Cm in tomato seeds involves bacterial isolation followed by molecular confirmation (qPCR) and pathogenicity test. A method based on the same principles can be used to detect X spp. on tomato and pepper seeds. This process is labor-intensive, requiring up to 12 days for results. Internationally, the ISHI-Veg method (ISF) incorporates as a pre-screen a qPCR directly on seed-extract without isolation (SE-qPCR), allowing faster results for negative samples (2 days). However, this method can yield false negatives on hypochlorite-treated seeds. Nowadays, the majority of tomato and pepper seeds are treated with hypochlorite before being imported into France. Consequently, seed lots imported into France cannot be tested by the SE-qPCR method.

The aim of this collaborative project is to develop an improved SE-qPCR pre-screening test for detecting Cm and X. spp., which can be applied to hypochlorite-treated seeds with the same reliability as to untreated seeds. An additional goal is to harmonize the maceration and DNA extraction steps to detect all five pathogens from a single seed extract.

Approximately 95% of seed samples are negative for these pathogens (GEVES data 2022-2023). The application of SE-qPCR would significantly reduce testing time while maintaining reliability. All the work accomplished will enable SE-qPCR to be included in the official French method for controlling bacterial diseases.

References:

Development of a real-time PCR method for the identification of cucurbit seed-borne Pseudomonas syringae species

Mylène RUH (1), Quentin BEAUREPERE (1), Marion JOUSSELIN (1), Amélie BALOCHE (1), Leydi MARTIN (1), Nicolas DENANCE (1), Isabelle SERANDAT (1), Jaiana MALABARBA (1) and Amandine LE VAN (1)

1: GEVES (Variety and Seed Study and Control Group), 25 rue Georges Morel, 49071 Beaucouzé, France

Pseudomonas syringae species are responsible for diseases that are a significant threat to cucurbit crops worldwide, like Vein Clearing on Zucchini (VCZ) caused by several lineages of P. syringae (Manceau et al., 2011; Lacault et al., 2020) and Angular Leaf Spot (ALS) caused by P. syringae pv. lachrymans and infecting all cucurbit crops (Olczakwoltman et al., 2009; Bhat et al., 2010). These are seed-borne pests, for which detection in seed samples is essential to limit their spread. Current GEVES tests are based on isolation of suspect colonies on a nutrient medium, followed by identity confirmation by end-point PCR and/or pathogenicity assay. All these tests are completed in 36 days, including around 20 to confirm identity. A faster and still accurate identification method would therefore be appropriate for the effective management of these diseases. We present here a real-time PCR method to confirm the identity of VCZ and ALS strains in just around a week with a single analysis. Strains representing the genetic diversity of targeted and closely related species were characterised by biochemical and pathogenicity tests, PCRs (Manceau et al., 2011; Lacault et al., 2023), and phylogenetic analyses. Primers and probe designed for ALS strains and 4Ba primers and probe for VCZ strains (Lacault et al., 2023) were tested on the characterised strains to validate the method's analytical specificity and additional analyses were carried out to validate the repeatability and reproducibility of the method. In conclusion, we can use this real-time PCR method to confirm the identity of suspect colonies in alternative to the end-point PCR and pathogenicity test. Moreover, these validated primers and probes could be used for the development of a new SE-qPCR pre-screening method to detect sequences of VCZ and ALS strains directly from seed macerates without isolation on media.

References:

Bhat, N.A., Bhat, K.A., Zargar, M.Y., Teli, M.A., Nazir, M., Zargar, S.M. (2010). Current status of angular leaf spot (Pseudomonas syringae pv. lachrymans) of cucumber: a review. International Journal of Current Research 8, 1–11.

Lacault, C., Briand, M., Jacques, M. A., and Darrasse, A. (2020). Zucchini Vein Clearing Disease Is Caused by Several Lineages Within Pseudomonas syringae Species Complex. Phytopathology 110, 744-757.

Lacault, C., Briand, M., Jacques, M. A., and Darrasse, A. (2023). Development of tools to detect and identify strains belonging to the Pseudomonas syringae species complex responsible for vein clearing of zucchini. bioRxiv 2023.05.02.539078.

Manceau, C., Gironde, S., Briand, B., and Lybeert, H. (2011). Means and methods for detecting and identifying a novel bacterium responsible for phytosanitary disorders in plants (zucchini) and novel resistant plants. Patents, #WO2011003984, World Intellectual Property Organization, Geneva, Switzerland.

Olczakwoltman, H., Schollenberger, M., Niemirowicz-Szczytt, K. (2009). Genetic background of host-pathogen interaction between Cucumis sativus L. and Pseudomonas syringae pv. lachrymans, Journal of Applied Genetics 50 (1), 1–7.

PROPHYLE, a new experimental and analytical platform

Odile BERGE

INRAE, UR0407 Pathologie végétale, Avignon, France

PROPHYLE is an experimental and analytical platform attached to UR0407 Plant Pathology INRAE in Avignon, which focuses on emerging crop problems in order to better characterize the associated plant pathogens. It comprises three technology divisions: experimental facilities, etiology and microscopy. It supports the unit's thematic areas within the framework of research projects.

PROPHYLE is also open to the scientific community as a whole, and to all public or private partners wishing to benefit from its technologies and facilities, via services.

References:

https://eng-pathologie-vegetale.paca.hub.inrae.fr/infrastructures/prophyle

What is the link between T6SS-mediated interference competition and exploitative competition within seed microbiome ?

Tiffany GARIN, Agathe BRAULT, Coralie MARAIS, Martial BRIAND, Anne PREVEAUX, Marie SIMONIN, Matthieu BARRET, Alain SARNIGUET (all 1)

1Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, F-49000 Angers, France

It is commonly established that competition for resources drives microbiome assemblage and could be harnessed to limit pathogen invasion. However interference competition mediated by toxic compounds is another driver but based on diffusible molecules and high cell density. Alternative contact-dependant antibiosis like Type VI Secretion System (T6SS) provides efficient killing of competitors at low cell density. We ask how T6SS is complementary to exploitative competition and drives seed microbiome. For this we described the range of targets of the T6SS of Stenotrophomonas rhizophila (1) within bacterial communities in vitro and in planta. We demonstrated that T6SS shaped seed bacterial communities and their transmission to seedlings. We also observed that the most competitive bacteria for resources were also the most sensitive to the T6SS of S. rhizophila. We conclude to a strong synergy of exploitative and contact-dependant interference competitions (2).

References:

(1)Garin T, Brin C, Préveaux A, Brault A, Briand M, Simonin M, Barret M, Journet L, Sarniguet A. 2024. The type VI secretion system of Stenotrophomonas rhizophila CFBP13503 limits the transmission of Xanthomonas campestris pv. campestris 8004 from radish seeds to seedlings. Molecular Plant Pathology 25:e13412. https://doi.org/10.1111/mpp.13412.

(2)GarinT, Brault A, Marais C, Briand M, Préveaux A, Simonin M, Barret M, Sarniguet A.T6SS-mediated competition by Stenotrophomonas rhizophila shapes seed-borne bacterial communities and seed-to-seedling transmission dynamics bioRxiv, 2024. https://doi.org/10.1101/2024.07.22.604635

List of participants

Nom	Prénom	email
Achouak	Wafa	wafa.achouak@cea.fr
Agert	Laura	laura.agert@inrae.fr
Alunni	Benoît	benoit.alunni@inrae.fr
Amaro-Lauer	Coline	coline.amaro@i2bc.paris-saclay.fr
Arroyo Velez	Noe	Noe.Arroyo-Velez@inrae.fr
Arsene-Ploetze	Florence	florence.ploetze@ibmp-cnrs.unistra.fr
Audran	Corinne	corinne.audran@inrae.fr
Auguy	Florence	florence.auguy@ird.fr
Balleux	Guillaume	guillaume.balleux@uliege.be
Barbier	Michel	michel.barbier@ird.fr
Barbier	Marion	marion.barbier@inrae.fr
Barnard	Romain	romain.barnard@inrae.fr
Barny	Marie-Anne	marie-anne.barny@sorbonne-universite.fr
Barret	Matthieu	matthieu.barret@inrae.fr
Baud	Anaelle	anaelle.baud@univ-lyon1.fr
Becker	Nathalie	nathalie.becker@mnhn.fr
Béna	Gilles	gilles.bena@ird.fr
Berge	Odile	odile.berge@inrae.fr
Bertolla	Franck	franck.bertolla@univ-lyon1.fr
Bilesky Jose	Natalia	nataliabilesky@hotmail.com
Biondi	Emanuele	emanuele.biondi@i2bc.paris-saclay.fr
Blasco	Camille	camille.blasco@ird.fr
Blonde	Clara	clara.blonde@insa-lyon.fr
Boubakri	Hasna	hasna.boubakri@univ-lyon1.fr
Bouche	Ségolène	segolene.bouche@inrae.fr
Boulanger	Alice	alice.boulanger@inrae.fr
Boutigny	Anne-Laure	anne-laure.boutigny@anses.fr
Boutron	Eve	boutron.eve@gmail.com
Bragard	Claude	claude.bragard@uclouvain.be
Brottier	Laurent	laurent.brottier@ird.fr
Burkhardt	Nicolas	nicolas.burkhardt@inrae.fr
Cabre	Lisa	lisa.cabre@roullier.com
Caddeo	Amélie	amelie.caddeo@imean-biotech.com
Caly Simbou	Eva	evacalys@gmail.com

Camuel	Alicia	camuel.alicia@gmail.com
Capela	Delphine	delphine.capela@inrae.fr
Cellier	Gilles	gilles.cellier@anses.fr
Cesbron	Sophie	sophie.cesbron@inrae.fr
Chadelaud	Thomas	thomas.chadelaud@inrae.fr
Chargy	Manon	manon.chargy@inrae.fr
Cheminat	Margaux	margaux.cheminat@etu.unistra.fr
Chen	Nicolas	nicolas.chen@institut-agro.fr
Chesneau	Guillaume	gchesneau@mpipz.mpg.de
Cigna	Jérémy	jeremy.cigna@inov3pt.fr
Cociancich	Stéphane	stephane.cociancich@cirad.fr
Colaert-Sentenac	Louna	louna.colaert-sentenac@inrae.fr
Condemine	Guy	guy.condemine@insa-lyon.fr
Cunty	Amandine	amandine.cunty@anses.fr
Czernic	Pierre	pierre.czernic@umontpellier.fr
Dagva	Oyut	oyut.dagva@insa-lyon.fr
Darrasse	Armelle	armelle.darrasse@inrae.fr
Daviddi	Naïs	nais.daviddi@universite-paris-saclay.fr
De Zélicourt	Axel	axel.de-julien-de-zelicourt@universite-paris-saclay.fr
Degrave	Alexandre	alexandre.degrave@agrocampus-ouest.fr
Dendene	Sara	sara.dendene@i2bc.paris-saclay.fr
Desbrosses	Guilhem	guilhem.desbrosses@umontpellier.fr
Deveau	Aurélie	aurelie.deveau@inrae.fr
Diab	Emtinan	e.a.e.diab@biology.leidenuniv.nl
Dittmer	Jessica	jessica.dittmer@inrae.fr
Dolly	Maurane	maurane.dolly@i2bc.paris-saclay.fr
Doré	Jeanne	jeanne.dore@univ-lyon1.fr
Drogue	Quentin	quentin.drogue@roullier.com
Dubois	Quentin	quentin.dubois@insa-lyon.fr
Duclercq	Jerome	jerome.duclercq@u-picardie.fr
Dufour	Marie-Cécile	marie-cecile.dufour@inrae.fr
Fernandes Hertel	Mariana	mariana.fernandes-hertel@cea.fr
Fischer-Le Saux	Marion	marion.le-saux@inrae.fr
Fochesato	Sylvain	sylvain.fochesato@cea.fr
Folletti	Tifaine	tifaine.folletti@inrae.fr

Foucher	Justine	justine.foucher@geves.fr
Fournier	Paola	paola.fournier@inrae.fr
Frantz	Axelle	axelle.frantz@inrae.fr
Gagnevin	Lionel	lionel.gagnevin@cirad.fr
Girard	Olivier	o.girard@tempcontrol.fr
Gourion	Benjamin	benjamin.gourion@inrae.fr
Grosselin	Marie	marie.grosselin@eu.goldstandarddiagnostics.com
Gueguen	Erwan	erwan.gueguen@univ-lyon1.fr
Guidot	Alice	alice.guidot@inrae.fr
Halter	Thierry	thierry@immunrisetechnologies.com
Helman	Yael	yael.helman@mail.huji.ac.il
Henry	Gwennaelle	gwennaelle.henry@univ-lyon1.fr
Hillairet	Erin	erin.hillairet@inrae.fr
Hommais	Florence	florence.hommais@univ-lyon1.fr
Houdinet	Gabriella	gabriella.houdinet@inrae.fr
Hutin	Mathilde	mathilde.hutin@ird.fr
Ilyas	Amina	amina.ilyas@universite-paris-saclay.fr
Jacques	Marie-Agnès	marie-agnes.jacques@inrae.fr
Jacquiod	Samuel	samuel.jacquiod@u-bourgogne.fr
Jobert	Léa	lea.jobert@ird.fr
Keita	Ibrahim	ibrahim.keita@inrae.fr
Koebnik	Ralf	koebnik@gmx.de
Labbé	Frédéric	frederic.labbe@cirad.fr
Lavire	Céline	celine.lavire@univ-lyon1.fr
Le Siou	Anaëlle	anaelle.lesiou@gmail.com
Lebeau	Liam	lebeau@bio.ens.psl.eu
Lepère	Gersende	gersende.lepere@ens.fr
Lheureux	Alex	alex.lheureux@eu.goldstandarddiagnostics.com
Martin	Valerie	valerie.martin@usu.edu
Marty	Soline	soline.marty@inrae.fr
Méndez-Bravo	Alfonso	alfonso.mendez-bravo@inrae.fr
Mirguet	Céline	celine.mirguet@inrae.fr
Moenne-Loccoz	Yvan	yvan.moenne-loccoz@univ-lyon1.fr
Mohammedi	Roza	roza.mohammedi@i2bc.paris-saclay.fr
Moutacharrif	Sara	sara.moutacharrif@insa-lyon.fr

Navarro	Marvin	marvin.navarro@inrae.fr
Nazaret	Fanny	fannynazaret@gmail.com
Noël	Alba	alba.noel@univ-angers.fr
Noël	Laurent	laurent.noel@inrae.fr
Palomares	Marie-Ange	marieange.palomares@gmail.com
Pécourt	Audrey	audrey.pecourt@u-picardie.fr
Peduzzi	Chloé	chloe.peduzzi@uclouvain.be
Peeters	Nemo	nemo.peeters@inrae.fr
Perez Quintero	Alvaro	alperezqui@gmail.com
Pesenti	Lena	lena.pesenti@uclouvain.be
Pivato	Barbara	barbara.pivato@inrae.fr
Portier	Perrine	perrine.portier@inrae.fr
Prigent-Combaret	Claire	claire.prigent-combaret@univ-lyon1.fr
Prudot D'Avigny	François	fprudot@gmail.com
Quibod	Ian-Lorenzo	ian_lorenzo.quibod@ird.fr
Quilbé	Johan	johanq@mbg.au.dk
Ratet	Pascal	pascal.ratet@cnrs.fr
Rendueles	Olaya	olaya.rendueles-garcia@univ-tlse3.fr
Reverchon	Frédérique	frederique.reverchon@inrae.fr
Rigolet	Augustin	a.rigolet@biology.leidenuniv.nl
Robene	Isabelle	isabelle.robene@cirad.fr
Routaboul	Jean-Marc	jean-marc.routaboul@inrae.fr
Roux	Chloé	chloe.roux@inrae.fr
Ruh	Mylène	mylene.ruh@geves.fr
Sallen	Antinéa	antinea.sallen@anses.fr
Sanchez	Lisa	lisa.sanchez@univ-reims.fr
Santaella	Catherine	catherine.santaella@cea.fr
Sarazin	Vivien	sarazinv@agrostation.fr
Sarniguet	Alain	alain.sarniguet@inrae.fr
Sauviac	Laurent	laurent.sauviac@inrae.fr
Schaller	Hubert	hubert.schaller@ibmp-cnrs.unistra.fr
Schikora	Adam	adam.schikora@julius-kuehn.de
Siby	Heimrick	heimrick.siby@roullier.com
Simonin	Marie	marie.simonin@inrae.fr
Stuttmann	Johannes	johannes.stuttmann@gmail.com

Suteau	Logan	logan.suteau@inrae.fr
Szurek	Boris	boris.szurek@ird.fr
Taillefer	Boris	boris.taillefer@inrae.fr
Terret-Welter	Zoé	zoe.terret-welter@hmclause.com
Thonon	Salomé	salome.thonon@uclouvain.be
Topp	Ed	ed.topp@inrae.fr
Torres-Barceló	Clara	clara.torresbarcelo@inrae.fr
Vacher	Corinne	corinne.vacher@inrae.fr
Vandelle	Elodie	elodiegenevieve.vandelle@univr.it
Vanhove	Terriane	terriane@hotmail.fr
Vial	Ludovic	ludovic.vial@univ-lyon1.fr
Vicente	Sylvain	sylvain.vicente@inrae.fr
Weisskopf	Laure	laure.weisskopf@unifr.ch
Wisniewski-Dyé	Florence	florence.wisniewski@univ-lyon1.fr
Zannis-Peyrot	Timothée	timothee.zannis-peyrot@univ-lyon1.fr
Zarate-Chaves	Carlos	carlos.zarate-chaves@ird.fr