7th IBWS

International Bacterial Wilt Symposium

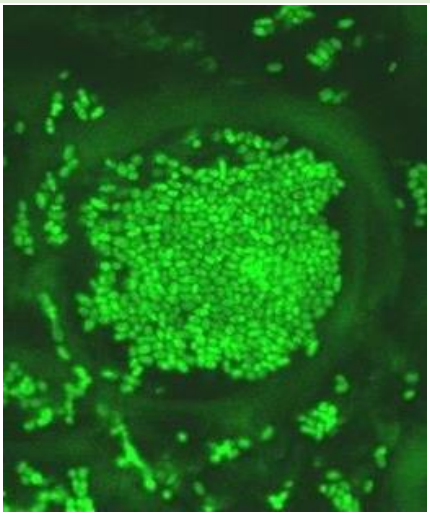
Montevideo – URUGUAY

7th International Bacterial Wilt Symposium

19 – 24 March 2023

Montevideo, Uruguay

ABSTRACT BOOK



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INIA

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Caitilyn Allen	University of Wisconsin-Mardison, United States of America
David Norman	University of Florida, United States of America
Boshou Liao	Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, China
Yasufumi Hikichi	Kochi University, Japan

INVITED SPEAKERS

Leonardo De La Fuente	Auburn University, United States of America
Gitta Coaker	University of California, Davis, United States of America
Adriana Bernal	Universidad de los Andes, Colombia
Jhonatan Jacobs	Ohio University, United States of America
Alberto Macho	Shangai Center for Plant Stress Biology, China
Anjali Iyer-Pascuzzi	Purdue University, United States of America
Francisco Vilaró	Institute of Agricultural Research (INIA) / Universidad de la República, Uruguay
Gilles Cellier	Anses, Plant Health Laboratory, La Reunion, France
Kalpana Sharma	International Potato Center, Kenya
Kenji Kai	Osaka Metropolitan University, Japan
Kornelia Smalla	Julius Kühn-Institut, Germany
Marco Dalla Rizza	Institute of Agricultural Research (INIA), Uruguay
Seon-Woo Lee	Dong-A University, Republic of Korea
Ville-Petri Friman	University of York, United Kingdom

SPONSORS

Welcome message

On behalf of all the members of the Organizing Committee from Universidad de la República (Udelar) and the National Agricultural Research Institute (INIA) of Uruguay, I would like to extend our warmest welcome to Montevideo for all participants of the 7th International Bacterial Wilt Symposium (7IBWS).

Bacterial wilt caused by plant pathogenic *Ralstonia* species is one of the most important diseases affecting the production of many important food crops. Because of its very broad host range and wide geographical distribution, it is arguably the world's single most harmful bacterial plant pathogen. A large scientific community has been dedicated to studying bacterial wilt diseases worldwide. Several International Bacterial Wilt Symposia have been organized in different locations across the world including Taiwan (1992), Guadeloupe (1997), White River (2002), York (2006), Wuhan (2011), and Toulouse (2016). Without any doubt, this event has become a reference meeting for the scientific community working on this relevant topic. We are delighted and proud to host the 7th IBWS edition in Uruguay, being the first time, it takes place in a South American country.

Just like the previous six editions, I am confident that the 7IBWS 2023 will play an important role in encouraging activities for development of bacterial wilt research. Partaking advances and innovative ideas, I hope that this event will promote collaborative research within our scientific community. The program of this event is broad and exciting, including both fundamental and applied research topics, as well as invited conferences focused on other related plant pathogenic bacteria. This framework provides a unique meeting ground for researchers spanning the whole spectrum of our discipline. We hope that you will have a productive and fun-filled time at this very special conference.

To put a conference of this magnitude together is not a small task. To this end, I would like to express my sincere gratitude to all the local institutions and organizing staff for their constant support. I would also like to thank all the invited speakers and the members of the scientific committee, for their presence and contributions for the reviewing process and planning of the scientific sessions. I would also like to recognize all the sponsoring organizations for providing their generous financial support, making possible to carry out this conference and to cover more than 20 travel awards for students and young researchers from all over the world. Lastly, we would like to thank all the conference participants for their contributions which are the foundations of this meeting.

Welcome to Montevideo and enjoy the conference!

General Conference Chair:

María Inés Siri

Program

SUN — MARCH 19 - José Luis Massera Building

16:00	Participant arrival and registration
17:30	Welcome words and musical show - Las Coralinas choir
18:30	Tango show and welcome reception

MON — MARCH 20 - José Luis Massera Building

08:00 – 08:30	Participant arrival and poster set up (Session A)
08:30 – 09:00	Opening Ceremony
Special session: "Insights and teachings from diverse plant-pathogenic bacteria" (part I) Chairs: Caitilyn Allen and Stephane Genin	
09:00 – 09:35	"*Xylella fastidiosa* is adapted to live exclusively in the xylem" – Leonardo De La Fuente
09:35 – 10:10	"Host specificity and immune recognition in *Clavibacter*-plant interactions" – Gitta Coaker
10:10 – 10:40	Morning break
Special session: "Insights and teachings from diverse plant-pathogenic bacteria" (part II) Chairs: Caitilyn Allen and Stephane Genin	
10:40 – 11:15	"New insights into the interaction between *Xanthomonas phaseoli* pv. *manihotis* and cassava – Adriana Bernal
11:15 – 11:50	"Along the same vein - defining the basis of *Xanthomonas* and *Ralstonia* plant colonization – Jonathan Jacobs
11:50 – 12:00	Final discussion and concluding remarks
12:00 – 12:40	Keynote lecture: "The special case of Race 3 biovar 2: Why is *Ralstonia solanacearum* IIB-1 so effective?" – Caitilyn Allen
12:40 – 14:00	Lunch break – Poster set up (Poster Session A)
Plenary Session: "Diversity, structure and evolution of the *Ralstonia solanacearum* species complex populations" Chairs: María Inés Siri and Myriam Valenzuela	
14:00 – 14:40	Opening lecture: "The *Ralstonia solanacearum* species complex in the age of epidemiology: exploration of its molecular diversity and population structure" – Gilles Cellier
14:40 – 14:55	Global biogeography and natural host range of pathogenic *Ralstonia* lineages – Tiffany Lowe-Power
14:55 – 15:10	*Ralstonia solanacearum* can rapidly evolve tolerance to volatile organic compounds produced by antagonistic bacteria – Raza Waseem
15:10 – 15:25	Adapting to environmental reservoirs is costly for the plant-pathogenic bacterium *Ralstonia solanacearum* – Evie Farnham
15:25 – 16:05	Closing lecture: "*Ralstonia solanacearum* species complex strains causing bacterial wilt of potato in sub-Saharan Africa: an impending socio-economic disaster" - Kalpana Sharma
16:05 – 16:40	Poster flash talks - Session A
16:40 – 18:00	Afternoon break – Exhibition and poster viewing (Session A)

TUE MARCH 21 - José Luis Massera Building

08:00 – 08:30	Participant arrival and poster set up (Session B)
Plenary Session: "Infection and virulence mechanisms" Chairs: Marc Valls and Yasufumi Hikichi	
08:30 – 09:10	Opening lecture: "Deciphering the activities of *Ralstonia solanacearum* type III effectors: beyond activation and suppression of immunity" – Alberto Macho
09:10 – 09:25	Let's stick together: mechanisms of host attachment and biofilm formation in bacterial wilt – Mariama Carter
09:25 – 09:40	Complex regulation of novel regulators TapV and CysB on expression of Type III Secretion System genes and pathogenicity in *Ralstonia solanacearum* – Yong Zhang
09:40 – 10:20	Closing lecture: "The Phc quorum sensing system in RSSC: specificity in signal production and response, regulation of secondary metabolism, and chemical control – Kenji Kai
10:20 – 10:50	Morning break
Plenary Session: "Mechanisms of plant-pathogenic *Ralstonia* interactions" Chairs: Anjali Iyer-Pascuzzi and Alberto Macho	
10:50 – 11:25	Host adaptation and pathogenesis of *Ralstonia pseudosolanacearum*: mechanisms and evolution – Stephane Genin
11:25 – 12:00	Contribution of the quorum sensing to infection in tomato roots and virulence in *Ralstonia pseudosolanacearum* strain OE1-1 – Yasufumi Hikichi
12:00 – 12:35	Restriction of *Ralstonia solanacearum* colonization in tomato resistant to bacterial wilt – Marc Valls
12:35 – 12:45	Final discussion and concluding remarks
13:00-14:00	Lunch break
Plenary Session: "Plant responses and disease development" Chairs: Virginia Ferreira and Liao Boshou	
14:00 – 14:40	Opening lecture: "Getting to the root of resistance to *Ralstonia solanacearum* in tomato" – Anjali Iyer-Pascuzzi (USA)
14:40 – 14:55	SA-independent mechanism in the tomato diageotropica (*dgt*) mutant enhance root-mediated resistance to *Ralstonia solanacearum* K60 – Katherine Rivera-Zuluaga
14:55 – 15:10	Virulence of novel *Ralstonia pseudosolanacearum* (phylotype I) isolates from rose, blueberry and mandevilla on seed potato – Maria Bergsma-Vlami
15:10 – 15:25	Epidemiology of Blood disease, an emerging threat to banana production – Jane Ray
15:25 – 16:00	Poster flash talks - Session B
16:00 – 17:30	Afternoon break – Exhibition and poster viewing (Session B)
17:30 – 17:45	Tribute to Philippe Prior
Hayward-Prior Award Session Chairs: Caitilyn Allen and Gilles Cellier	
17:45 – 18:00	Genomic and phenotypic diversity of *Ralstonia pseudosolanacearum* infecting multiple hosts in Cambodia – Taylor Klass
18:00 – 18:15	Multiple and overlapping chemoreceptors within *Ralstonia* species have diverging ligand specificities – Rebecca Schomer
18:15 – 18:30	Regulation of the micacocidin production-related gene RSc1806 and its involvement in virulence of *Ralstonia pseudosolanacearum* strain OE1-1 – Yuki Terazawa
18:30 – 18:45	Effect of bacterial wilt incidence on growth and yield response of tomato, potato and capsicum under various field soil amendments – Elizabeth Kariko Kago
18:45 – 19:00	Biological and molecular characterization of bacteriophages with biocontrol potential against bacterial wilt caused by *Ralstonia solanacearum* in tomato crops – Paulina Parra-Castro

WED MARCH 22

8:00-18:30	Guided tour to Punta del Este Optional activity, ticket not included in the registration fee

THU MARCH 23 – INIA Las Brujas Experimental Station

07:30	Departure from José Luis Massera building
08:30 – 09:00	Arrival to INIA Las Brujas Experimental Station – Welcome and introduction to INIA
09:00 - 9:40	Invited lecture: "Breeding for potato bacterial wilt resistance in Uruguay" – Francisco Vilaró
9:40 - 10:10	Morning break
Plenary Session: "Host resistance and crop improvement" Chairs: Miryam Valenzuela and Guillermo Galván	
10:10 – 10:50	Opening lecture: "Recent advances in the selection of potato clones resistant to bacterial wilt in Brazil" – Mauricio Rossato
10:50 – 11:05	Adding value to our genetic resources: characterization and evaluation of potato wild relatives from Uruguay for bacterial wilt resistance and other traits of interest for breeding – Paola Gaiero
11:05 – 11:20	Identification of molecular markers for resistance to bacterial wilt in peanut - Huaiyong Luo
11:20 – 11:35	Discovery of functional R-genes in resistant rabbiteye blueberry (*Vaccinium ashei*) against *Ralstonia solanacearum* – Ana María Bocsanczy
11:35 – 12:15	Closing lecture: "Effect of the EFR gene on potato-bacterial wilt interaction and first evaluation of agronomic behavior" – Marco Dalla Rizza
12:15 – 13:30	Lunch break and exhibition of local stands
Plenary Session: "Innovative control strategies and integrated management" Chairs: María Julia Pianzzola and Mauricio Rossato	
13:30 – 14:10	Opening lecture: "Ecology and evolution of phage-bacteria interactions in the rhizosphere: consequences for microbiome functioning and control of plant disease outbreaks" – Ville-Petri Friman
14:10 – 14:25	Detection and analysis of CRISPR locus in the *Ralstonia solanacearum* species complex – Cristofer Motoche
14:25 – 14:40	Pyramiding host resistance to bacterial wilt and other major soil-borne diseases for integrated management in peanut – Boshou Liao
14:40 – 14:55	Bioassay-based method for screening biological control agents against tobacco bacterial wilt – Can-Hua Lu
14:55 – 15:10	Integrated strategies to manage bacterial wilt (*Ralstonia*) of tomato in North Carolina, USA – Prem Magar
15:40 – 16:30	Afternoon break and exhibition of local stands
16:30	Return to Montevideo
17:30	Arrival to José Luis Massera building

FRI — MARCH 24 - José Luis Massera Building

Plenary Session: "Plant-pathogen interactions within the phytobiome" Chairs: Virginia Ferreira and David James Norman	
08:30 – 09:10	Opening lecture: "Bacterial wilt resistance and root microbiome in tomato" - Seon-Woo Lee
09:10 – 09:25	Transcriptional landscape of the *Ralstonia solanacearum* life cycle: identification of key genes for growth in soil – Mercedes Rocafort
09:25 – 09:40	Exposing phage to multiple host genotypes can improve outcome of phage training with phytopathogenic *Ralstonia solanacearum* – Sophie James
09:40 – 09:55	Indirect reduction of *Ralstonia solanacearum* via pathogen helper inhibition – Mei Li
09:55 – 10:35	Closing lecture: "Potential role of rhizosphere microbiome modulation in controlling *Ralstonia solanacearum* caused bacterial wilt- Kornelia Smalla
10:35 – 10:50	Closing ceremony
10:50 – 11:30	Morning break and farewell

Abstracts of Oral Presentations

Special Session:

"Insights and teachings from diverse plant-pathogenic bacteria"

Xylella fastidiosa is adapted to live exclusively in the xylem.

De La Fuente L.[1]

[1]Department of Entomology and Plant Pathology, Auburn University, Auburn, Alabama, USA.

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Xylella fastidiosa is a xylem-limited bacterial plant pathogen that is vectored only by sap-sucking insects. More than 650 plant species are known to be infected by this pathogen, although many of them do not develop symptoms. Pierce's disease in grapevines has been known in the USA for ~150 years, but in the last decades this and other emergent diseases caused by *X. fastidiosa* have been detected in other parts of the world. Although historically most reports were originated in the Americas, in the last 10 years it has been detected in Europe and Asia, notably causing the devastating olive quick decline syndrome in the south of Italy. During this presentation we will review the molecular and microbiological traits of this pathogen that allow it to live successfully inside the xylem vessels. Mineral elements such as calcium, copper and zinc were shown to manipulate growth and virulence of *X. fastidiosa*, and novel Zn nanoparticles are being investigated as possible antibacterial treatments. The process of natural competence, which is the ability of a bacterium to acquire and recombine pieces of DNA from the environment, is being studied for its potential role in niche adaptation and evolution of *X. fastidiosa*. One of the key bacterial structures for this process, type IV pili, was dissected by determining the function of each one of the 38 genes involved in its biosynthesis and regulation. Understanding the strategies used by *X. fastidiosa* to exclusively live inside the xylem will help inform approaches that can target vulnerable aspects of this pathogen for disease control.

Comparative genomics of *Clavibacter* pathogens reveals drivers of host range.

Stevens D.[1], Ramsing C.[1], Teper D.[2], and Coaker G.[1]

[1]Department of Plant Pathology, University of California, Davis USA; [2]Agricultural Research Organization, Volcani Institute, Israel.

Pathogens of the *Clavibacter* genus colonize the xylem vasculature and cause disease in a host-specific manner in both monocots and dicots. Despite their described narrow host range, genetic drivers of pathogen host range have been a longstanding question in *Clavibacter* biology. Secreted pathogen effectors can shape plant-pathogen interactions by shifting host susceptibility, facilitating pathogen colonization, and promoting disease. For *C. michiganensis* (*Cm*), the causal agent of bacterial canker on tomato, serine proteases and carbohydrate activating enzymes (CAZymes) effector families are required for pathogenicity on tomato. To investigate the prevalence of effector families in *Clavibacter*, we sequenced ~70 isolates from five pathogenic species. All pathogen genomes were compared, and species could be clustered based on groups of virulence genes, indicating certain effectors may be critical for the ability to cause disease. In particular, serine protease effectors were found to be highly conserved in *Clavibacter* species causing disease on Solanaceous hosts. Two *Cm* effector proteases are recognized in eggplant and tobacco. Deletion of these protease effectors enables *Cm* to cause disease on both plants. These data indicate *Clavibacter* effector proteases are capable of mediating pathogenicity on Solanaceous hosts.

Invited Conference

New insights into the interaction between *Xanthomonas phaseoli* pv. *manihotis* and cassava.

Zarate-Chaves C. A.[1], Castillo D.[2], Rodriguez C. J.[2], Bejarano D.[2], Medina C.[2], López C.[3], Jacobs J. M.[4], Koebnik R.[1], Szurek B.[1], Bernal A.[2]

[1]PHIM, Univ Montpellier, IRD, CIRAD, INRAE, Institut Agro, Montpellier, France ; [2]Laboratorio de interacciones moleculares de microorganismos agrícolas (LIMMA), Universidad de los Andes, Bogotá, Colombia; [3]Manihot Biotec, Departamento de Biología, Universidad Nacional de Colombia, Bogotá, Colombia; [4] Department of Plant Pathology, The Ohio State University, Columbus, OH, USA.

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Cassava is a staple crop with major importance for food security, feeding over 800 million people in Africa, Asia, and South America. Cassava bacterial blight (CBB) is the most important bacterial disease of this crop, causing significant yield losses under conducive environmental conditions. *Xanthomonas phaseoli* pv. *manihotis* (*Xpm*), the causal agent of this disease, uses type III effectors as the most important pathogenicity factors. We have investigated the functional role and sequence diversity of TALEs (Transcription Activator-Like Effectors) and found two discrete groups of TALEs in bacterial strains from a worldwide collection. Transcriptional analyses, combined with *in silico* binding site prediction, identified potential targets of these proteins in promoter regions within the cassava genome. We recently developed a CRISPR interference (CRISPRi) tool to downregulate all TALEs simultaneously in a given *Xanthomonas* strain. CRISPRi confirmed the *SWEET* genes as susceptibility factors in *Xpm* and suggested them as susceptibility factors for another *Xanthomonas* species that infects cassava, albeit in a non-vascular manner. In addition, we have identified candidate targets for Xops (Xanthomonas outer proteins) in cassava using a massive yeast two-hybrid screen coupled with Illumina sequencing. Many of the candidate targets are linked to the carbohydrate metabolism of cassava, and we have explored others with implications in defense responses. These results could be applied to classical breeding and genome editing approaches with the aim of generating broad-spectrum CBB-resistant plants.

Along the same vein - defining the basis of *Xanthomonas* and *Ralstonia* plant colonization.

Jacobs J. M., Butchacas J., Roman-Reyan V., Merfa M. V.

Department of Plant Pathology, The Ohio State University, Columbus, OH, USA; Infectious Diseases Institute, The Ohio State University, Columbus, OH, USA.

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Bacteria colonize almost all environments, but few associate with plants. *Xanthomonas* and *Ralstonia* bacteria represent two genera with broad plant host ranges and the ability colonize diverse plant tissues. The common ancestor of these two distant genera likely diverged over 1900 million years ago, but each currently share common traits for plant colonization of tissues including the water transporting xylem. These events resulted from more recent horizontal transfer of genes encoding secreted proteins for xylem and host association. This presentation will describe current research aiming to define the evolutionary history of these distant pathogens that promote xylem colonization and host association.

Invited Conference

The special case of Race 3 biovar 2: Why is *Ralstonia solanacearum* IIB-1 so effective?

Allen C.[1], Hamilton C. D.[1,2], Dewberry R. J.[1], Sharma P.[3] and Vinatzer B. A.[3]

[1]Department of Plant Pathology, University of Wisconsin-Madison USA; [2]Department of Microbiology and Immunology, University of British Columbia Canada; [3]School of Plant and Environmental Sciences, Virginia Tech University USA.

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R. solanacearum phylotype IIB-1 strains, also known as Race 3 biovar 2 (R3bv2), cause potato brown rot and tomato bacterial wilt in cool tropical highlands and occasionally in Eurasian temperate zones. But are all cool virulent strains R3bv2? Are all R3bv2 strains cool virulent? To improve regulation of threatening *R. solanacearum* strains independently of the R3bv2 designation, we built a robust phylogenetic tree based on whole genome sequences of >275 *R. solanacearum* strains. We also measured virulence and colonization ability of representative strains on tomato and potato at 22°C and 28°C to define the relationship between phenotypes and phylogeny. This combined approach revealed that cool virulence is a quantitative trait not limited to the R3bv2 subgroup. Nevertheless, epidemiological data indicate that a single clonal lineage of South American origin is responsible for the destructive potato brown rot pandemic. This rapidly spreading brown rot pandemic lineage (BRPL) can be identified by whole genome sequencing and has a unique identifier in the public LINbase. Host resistance is the best control for bacterial wilt, but resistance mechanisms of the widely used Hawaii7996 tomato breeding line are unknown. Concerningly, we found Hawaii7996 resistance is overcome by BRPL strain UW551. Unlike other *Ralstonia* strains, UW551 grew well in *ex vivo* xylem sap from *Ralstonia*-infected Hawaii7996 plants. Moreover, other *Ralstonia* strains could grow in sap from Hawaii7996 plants previously infected by UW551. Thus, UW551 breaks Hawaii7996 resistance in part by detoxifying inhibitors in xylem sap. Metabolomics showed that sap from *Ralstonia*-infected Hawaii7996 contained abundant phenolic compounds, which are known plant antimicrobial defenses. Culturing UW551 in this sap reduced total phenolic levels, indicating that the resistance-breaking BRPL *Ralstonia* strain degrades these chemical defenses. Together, these results show that Hawaii7996 tomato wilt resistance depends in part on inducible phenolic compounds in xylem sap.

Plenary Session:

"Diversity, structure and evolution of the Ralstonia solanacearum species complex populations"

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

Cellier G.[1], Pecrix Y.[2]

[1]Plant Health Laboratory, Anses, Saint Pierre, Reunion Island; [2]CIRAD, UMR Peuplements végétaux et bioagresseurs en milieu tropical, Saint Pierre, Reunion Island.

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Long-distance traveling of human beings and movement of goods drastically increase over time in relation with 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 than sequence data alone, phylogenetic and epidemiological techniques are often use. Bacterial lineage-centered molecular genotyping techniques, such as multilocus variable number of tandem repeats analysis (MLVA), or whole genome sequencing (WGS) techniques 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 pathogen, such as the *Ralstonia solanacearum* species complex (RSSC), several studies achieved molecular characterization of outbreak strains during the last decade mostly using multiplex PCR phylotyping, *egl* partial sequencing, multilocus sequence typing (MLST), MLVA and WGS.
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 then review some key concepts in phylogenetic applied on molecular data associated with the RSSC during this last decade.

Invited Conference

Ralstonia solanacearum species complex strains causing bacterial wilt of potato in sub-saharian Africa: an impeding socio-economic disaster.

Sharma K.[1,2]

[1]CGIAR Research Program on Roots, Tubers and Bananas (RTB), International Potato Center (CIP), Nairobi, Kenya, [2]CGIAR Research Program on Roots, Tubers and Bananas (RTB), International Potato Center (CIP), Lima, Peru

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Potato is a staple food and a major source of household income in Sub-Saharan Africa (SSA). Despite its importance, yields remain low due to lack of proper agronomic practices, inadequate supply and use of high-quality seeds, and pests and diseases. In particular, Bacterial Wilt (BW) caused by *Ralstonia solanacearum* species complex (RSSC) strains is an emerging threat to potato production in SSA. BW, once established in field, is one of the most difficult diseases to manage, largely due to the nature of the pathogen being soil, seed and water borne. Informal seed system and the use of latently infected seed are the major reasons for RSSC spread and introduction of *Ralstonia* into their smallholdings. BW has become very widespread in SSA- it was detected from 158 of 263 in Ethiopia, 128 of 176 farms in Kenya, 62 of 104 farms in Rwanda and 166 of 228 surveyed farms in Uganda, resulting in 30-100% yield losses. Genetic diversity and distribution of RSSC strains from these countries were identified, and then isolates with the same sequevar were further analysed by multi-locus MLVA typing schemes. In Ethiopia, all of the RSSC strains were identified as Phylotype II sequevar 1, whereas in Uganda, 80% of strains were identified as Phylotype II sequevar 1, followed by Phylotype I sequevar 31 (18.5%) and phylotype III (1.5%). Kenyan samples were identified as phylotypes I (25%) and II (75%). Finding of phylotypes I in Kenyan and Ugandan highlands indicates that earlier recommendations for crop rotation as a management strategy may not be working as Phylotype I strains have a much wider host range and are able to survive better on alternative hosts including weeds. VNTR profiling of these strains suggested that Phylotype II sequevar 1 strains play an important epidemiological role in BW of potato and likely being disseminated via latently infected seed. Additional sampling of the pathogen from neighbouring countries would provide a clearer population structure of RSSC strains, map and trace the movement of epidemiological RSSC strains causing bacterial wilt of potato in SSA to provide evidence-based recommendations for policy makers on seed movement.

O1

The global biogeography and natural host range of pathogenic *Ralstonia* lineages.

Lowe-Power T.[1], Avalos J.[1], Bai Y.[1], Charco Munoz M.[1], Chipman K.[1], Tom C. E.[1], and D. Williams[1]

[1]Department of Plant Pathology, University of California Davis, USA

The *Ralstonia* species complex is a group of genetically diverse plant wilt pathogens with a global impact on plant health. Our goal is to create a "Global Ralstonia Diversity" database that contains the reported global distribution and host range of *Ralstonia* clades (e.g. phylotypes and sequevars). Working with a team of undergraduate students, we have initiated a literature meta-analaysis and we have collected location, host plant, and phylogenetic lineage metadata for nearly 8,000 strains from 105 geographic regions. We are in the process of developing a "Dashboard" that shows maps of Ralstonia lineages' distributions and graphical summaries of host range.

The aggregated data suggest that the pandemic brown rot lineage (IIB-1) is the most widely dispersed lineage, which was expected. The pandemic IIB-1 lineage has been isolated from more locations than Phyl. I in aggregate. The Phyl IIB-4 lineage has a broad geographic distribution across multiple continents. Although Phyl. III and IV are predominantly restricted to their geographic origins (Africa and Indonesia/Japan, respectively), strains from these phylotypes have been reported in the Americas (Phyl. III) and Eastern Africa (Phyl. IV).

The cumulative host range for plant pathogenic *Ralstonia* encompasses at least 392 plant species. Phylotype I has the broadest cumulative host range (95 species), followed by phylotype II (63 species). Phylotype III and IV have been isolated from significantly fewer plant species (7-16 species). The IIB-4 lineage has the broadest known host range of 48 plant species across many botanical families.

To improve the accessibility of RSSC phylogenomics, we have created an open science resource. We associated strain metadata (host of isolation, location of isolation, and clade) with over 250 genomes in public Kbase Narratives. KBase is a free, cloud-based bioinformatic analysis platform that will allow Ralstonia researchers to assemble genomes and identify the phylogenetic position of newly sequenced strains.

O2

Ralstonia solanacearum can rapidly evolve more tolerant to volatile organic compounds produced by antagonistic bacteria.

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The bacterial wilt pathogen, *Ralstonia solanacearum* (*RS*), has to compete with bacteria, fungi and other microbes in the soil before infecting plants. One-way microbes can suppress RS growth is through volatile organic compounds (VOCs) that can diffuse over long distances. VOCs produced by soil bacteria have been shown to exert plant pathogen biocontrol potential owing to their strong antimicrobial activity. While the impact of VOCs on soil microbial ecology is well established, their effect on plant pathogen evolution is yet poorly understood. Here we investigated the phenotypic and genetic basis of RS adaptation to VOCs produced by a biocontrol strain *Bacillus amyloliquefaciens* T-5 for approximately 550 bacterial generations at three VOC levels that suppressed the RS growth by 15% (low), 30% (intermediate) and 55% (high), respectively. After the experiment, we isolated 18 *RS* clones from each control (no VOC) and high-level VOC treatments and sequenced their whole genomes. We found that VOC selection led to a clear increase in VOC-tolerance, which was accompanied with cross-tolerance to several antibiotics commonly produced by soil bacteria. The increasing VOC-tolerance led to trade-offs with *RS* virulence, resulting in almost complete loss of pathogenicity *in planta*. At the genetic level, these phenotypic changes were associated with parallel mutations in genes encoding lipopolysaccharide O-antigen (*wecA*) and type-4 pilus biosynthesis (*pilM*), which both have been linked with outer membrane permeability to antimicrobials and plant pathogen virulence. Reverse genetic engineering revealed that both mutations were important, with *pilM* having a relatively larger negative effect on the virulence, while *wecA* having a relatively larger effect on increased antimicrobial tolerance. Together, our results suggest that microbial VOCs are important drivers of bacterial evolution and could potentially be used in biocontrol to select for less virulent pathogens via evolutionary trade-offs.

O3

Adapting to environmental reservoirs is costly for plant-pathogenic bacterium *Ralstonia solanacearum.*

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In the United Kingdom (UK), *Ralstonia solanacearum* has an established population within river water and riparian weed host (*Solanum dulcamara*) environments, causing occasional outbreaks with potato. Here, we combined comparative analysis of environmental samples with experimental evolution to investigate *R. solanacearum* diversity and underlying drivers of adaptation within the UK environment. We first phenotyped and genotyped almost 200 *R. solanacearum* isolates from the UK spanning three decades, since the first reported case in 1992. Based on 46 independent phenotypic traits, we found three distinct ecotypes within the population, each differing in their growth in nutrient limited conditions, trait generalism and specialism, and tolerance to extreme pH and salinity. Interestingly, this phenotypic variation was not explained by core genome differences between the ecotypes. To directly test if environmental selection pressures can drive *R. solanacearum* adaptation, we experimentally evolved a UK isolate under different pH and salinity stresses in the lab. We found that while *R. solanacearum* adapted to acidic and alkaline stresses, this was constrained by the addition of salinity. Furthermore, all adaptations were costly, leading to trade-offs between stress tolerance and metabolic capacity. Like our comparative genomic analysis, genome sequencing showed little core genome variation. However, the movement of insertion sequences (IS) was frequent, especially in the megaplasmid, and are associated with key virulence genes (endoglucanase precursor and type II secretion proteins) and enzymes involved in metabolism (minor extracellular protease and thioesterase). Together, these results suggest that environmental stress can drive *R. solanacearum* adaptation by triggering IS movement within bacterial genomes, which could potentially explain phenotypic differences we observed between ecotypes among the UK *R. solanacearum* population. Overall, rapid evolution within environmental reservoirs can affect *R. solanacearum* diversity, potentially affecting pathogen distribution and disease epidemiology in the UK.

Travel Award

Plenary Session:

"Infection and virulence mechanisms"

Deciphering the activities of *Ralstonia solanacearum* type III effectors: beyond activation and suppression of immunity.

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Ralstonia solanacearum relies on its type-III secreted effectors (T3Es) to cause disease. Given their importance and their broad spectrum of targets and biological activities, such T3Es can also be used as probes to discover new plant functions associated to biotic stress. T3Es have been shown to suppress immunity using multiple different strategies, but also to target plant physiological functions that allow bacteria to shape the niche into a favorable environment for bacterial proliferation. Moreover, *R. solanacearum* is known to employ other unique strategies to evade plant immunity, and therefore it is essential to improve our understanding of these strategies in order to generate sustainable resistance against bacterial wilt disease in crops. In this talk, I will give an overview of the strategies followed in our group aimed at understanding alterations in plant signalling, metabolism, and hormonal balances, mediated by *R. solanacearum* T3Es, underlying both immune responses and bacterial virulence activities. I will also describe in more detail specific T3E activities targeted to the manipulation of plant metabolism in order to support bacterial proliferation.

Invited Conference

The *phc* quorum sensing system in RSSC: specificity in signal production and response, regulation of secondary metabolism, and chemical control.

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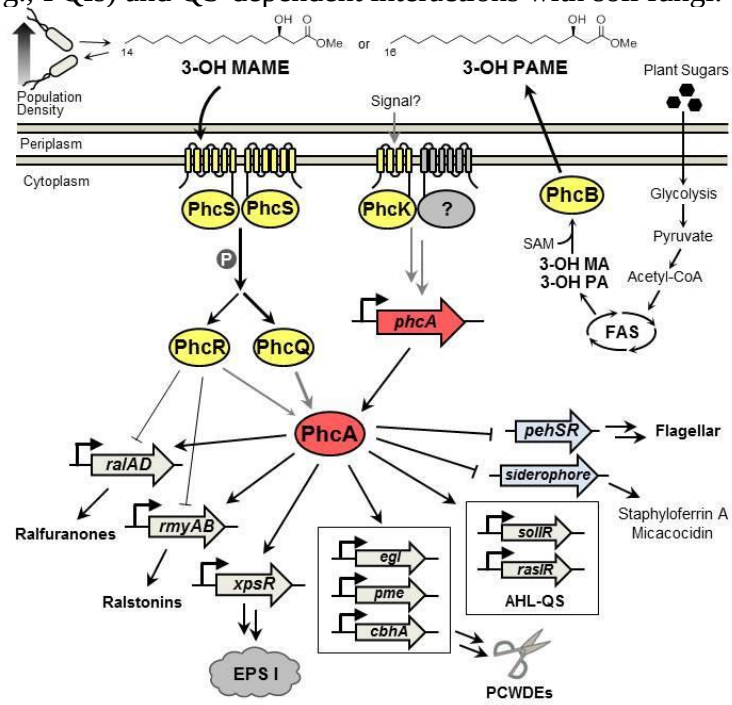
Ralstonia solanacearum species complex (RSSC) strains are devastating plant pathogens distributed worldwide. The *phc* quorum sensing (QS) is the primary cell density-dependent gene expression system in RSSC strains. RSSC strains use the QS system at each infection step to successfully move from the soil to the host stem. The *phc* QS regulates the expression of about 30% of all genes, which are related to cellular activity, primary and secondary metabolism, pathogenicity, and more. The *phc* regulatory elements encoded by the *phcBSRQ* operon (QS signal synthase and two-component system) and *phcA* (global virulence regulator) gene play vital roles. RSSC strains use methyl 3-hydroxymyristate (3-OH MAME) or methyl 3-hydroxypalmitate (3-OH PAME) as the QS signal. Each type of RSSC strain has specificity in generating and receiving its QS signal, but there may be no significant differences in signaling pathways between each other. The genetic and biochemical factors involved in the QS signal input and the regulatory network are presented. I would like to make some suggestions for future RSSC's QS research issues. Also, I summarized the current topics; control of the *phc* QS system (e.g., PQIs) and QS-dependent interactions with soil fungi.



Kai K. The *phc* quorum sensing system in *Ralstonia solanacearum* species complex. ***Annu. Rev. Microbiol.*** in press (2023). https://doi.org/10.1146/annurev-micro-032521-030537

O4

Let's stick together: mechanisms of host attachment and biofilm formation in bacterial wilt.

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Adhesins or adhesive proteins help bacteria stick to and colonize diverse surfaces and often contribute to virulence. During tomato stem colonization by *Ralstonia pseudosolanacearum (Rps)* strain GMI1000, adhesin genes *radA*, *rcpA*, and *rcpB* were upregulated in a ⊗*phcA* quorum sensing mutant while the sugar-binding lectin genes *lecF*, *lecM*, and *lecX* were strongly repressed. Based on this differential gene expression, we hypothesized that adhesins negatively regulated by PhcA contribute to early host-bacterium interactions when *Rps* experiences a low cell density, while those positively regulated by PhcA aid in host colonization and biofilm formation later in disease. During tomato root colonization *Rps* upregulated *rcpA* and *rcpB* relative to bacteria colonizing the stem, but not *radA*. All three lectins were upregulated during colonization of the root endosphere and stem in comparison to in the rhizoplane, the site of earliest host-pathogen interactions. Root colonization assays and confocal microscopy with Δ*rcpA/B* and Δ*radA* mutants revealed that all three adhesins help *Rps* attach to tomato roots but had no detectable role in root endosphere colonization. Additional mutant studies found that neither LecF, a fucose binding lectin, nor LecX, a xylose binding lectin, were required for root endosphere colonization, but LecF did contribute to colonization of the stem. Biofilm assays on abiotic surfaces found that *Rps* does not require RadA, RcpA, or RcpB for interbacterial attachment, but these proteins were essential for anchoring bacterial aggregates to a surface. The Δ*lecF* mutant produced about 25% less biofilm than wild-type GMI1000, while interestingly a Δ*lecX* mutant made twice as much biofilm as wild-type. Expression of *lecF* is increased in Δ*lecX* relative to wild-type, offering a possible explanation for its surprising hyper-attachment phenotype. Together, our results reveal an infection stage-specific deployment of diverse proteins for *Rps* adhesion, cohesion, and tomato colonization.

O5

Complex regulation of novel regulators TapV and CysB on expression of Type III Secretion System genes and pathogenicity in *Ralstonia solanacearum*

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A syringe like type III secretion system (T3SS) plays essential roles in pathogenicity of *Ralstonia solanacearum*, a causal agent of bacterial wilt disease on many plant species worldwide. Here, we genetically characterized involvement of two novel regulators of TapV and CysB, which were annotated as putative Type IV pili (T4P) assembly protein TapV (RSc1986 in GMI1000) and a CysB regulator (RSc2427 in GMI1000) controlling cysteine synthesis, respectively, on expression of the T3SS genes and pathogenicity. TapV was confirmed to be essential for some T4P-dependent properties, including twitching motility, swimming motility and host root adhesion, while *tapV* mutant produced more biofilm than the wild-type strain, which was different from known T4P mutants. CysB was confirmed to be essential for cysteine synthesis by positively regulating expression of genes for *cysU* and *cysI* regulons that control cysteine synthesis. Our gene expression studies revealed that both TapV and CysB were required for expression of the T3SS genes both *in vitro* and *in planta*. Involvement of TapV on the T3SS expression was mediated through PhcA-TapV-PrhG-HrpB pathway, but was T4P independent, while involvement of TapV on the T3SS was mediated through PrhG to HrpB pathway, but was independent of some other known regulators and growth deficiency under nutrient limited conditions. On the other hand, deletion of *tapV* significantly impaired abilities to migrate into and colonize host xylem vessels, but no alteration on intracellular proliferation in tobacco leaves, which was consistent as that of T4P mutants. Deletion of *cysB* significantly impaired exopolysaccharide production and swimming motility. All of these abilities contribute jointly to host colonization and infection process of *R. solanacearum* toward host plants. It provide novel insights into understanding of various biological functions of TapV and CysB regulators and complex regulatory networks on the T3SS in *R. solanacearum*.

Plenary Session:

"Mechanisms of plant-pathogenic Ralstonia interactions"

Host adaptation and pathogenesis of *Ralstonia pseudosolanacearum*: mechanisms and evolution.

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The bacterial wilt disease caused by the *R. pseudosolanacearum* GMI1000 strain is concomitant with a very high multiplication of the bacteria in the xylem vessels. We determined which nutritional sources of xylem sap allow the bacteria to rapidly reach high population levels. The metabolic activity of GMI1000 is largely controlled by central virulence regulators such as *phcA*. Through a combination of sequencing, metabolic network modeling, mutant generation and high-throughput phenotypic assays on 11 strains representative of the *R. solanacearum* species complex diversity, we showed the architecture of the metabolic network is globally conserved among strains, as well as the *phcA*-dependent control of several nutrient assimilation pathways.
In order to better understand the evolutionary mechanisms at work when the bacterium grows in the xylem of plants, we set up several years ago an evolution experiment of strain GMI1000 by serial passages from plant to plant to select better adapted individual clones. We now have an atlas of genetic mutations/alterations associated with *in planta* fitness gains of the clones carrying them, as well as the transcriptomic signatures of these evolved clones. We also identified clones with significant fitness gains although no genetic changes could be identified but differences in methylation patterns are observed. Functional analyses have been started to study the role of these epigenetic modifications in the regulation of bacterial phenotypes and host adaptation.

Invited Conference

Contribution of the quorum sensing to infection in tomato roots and virulence in *Ralstonia pseudosolanacearum* strain OE1-1.

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Ralstonia pseudosolanacearum strain OE1-1, secretes methyl 3-hydroxymyristate (3-OH MAME) as a quorum sensing (QS) signal. The LysR family transcriptional regulator PhcA regulates QS-dependent genes responsible for QS-dependent phenotypes including virulence. The RSSC invades intercellular spaces of tomato roots through wounds where secondary roots emerge, progressing through intercellular spaces and eventually infecting xylem vessels to spread up into the stem through the xylem vessels. However, infection route of RSSC, which invades tomato roots through the root apex, to xylem vessels in depth and function of QS on infection route of RSSC remained unknown. To analyze behavior of the strain OE1-1 invaded through the root apexes in tomato roots, we first developed an *in vitro* pathosystem, using 4-day-old tomato seedlings with roots of approximately 20 mm in length without secondary roots, which were co-incubated with the strain OE1-1. The microscopic observation of toluidine blue-stained longitudinal semi-thin resin sections of tomato roots allowed to detect attachment of the strain OE1-1 to surfaces of the meristematic and elongation zones. We then observed colonization of OE1-1 in intercellular spaces between the epidermis and cortex in the elongation zone. The strain OE1-1 next infected cell wall-degenerated cortical cells and formed mushroom-shaped biofilms to progress through intercellular spaces of the cortex and endodermis, infecting pericycle cells and xylem vessels. The deletion of QS-induced plant cell wall-degrading enzymes (PCDWEs) secreted via the type II secretion system (T2SS), led to a reduced infectivity in cortical cells. Furthermore, the QS-deficient and T2SS-deficient mutants lost their infectivity in cortical cells and the following infection in xylem vessels. Taking together, infection of the strain OE1-1, which attaches to surfaces of the meristematic and elongation zones, in cortical cells in the elongation zone, dependently on QS-induced PCDWEs secreted via the T2SS, leads to its subsequent infection in xylem vessels.

Factors restricting *Ralstonia solanacearum* colonization in bacterial wilt resistant tomato plants.

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Ralstonia solanacearum is a devastating bacterial vascular pathogen causing bacterial wilt. In the field, resistance against this disease is quantitative and only available for breeders in tomato and eggplant.
To understand the basis of bacterial wilt resistance in tomato, we have used grafting of susceptible and resistant varieties and live monitoring reporter strains to investigate the spatio-temporal bacterial colonization dynamics. Our work reveals four different restrictions to the bacterium in resistant tomato: root colonization, vertical movement from roots to shoots, circular vascular bundle invasion and radial apoplastic spread in the cortex. We demonstrate that structural constraints to bacterial spread are key for tomato resistance to bacterial wilt and that this resistance is expressed both in root and shoot tissues. We also show that *R. solanacearum* is not only a vascular pathogen but spreads "out of the xylem", occupying the plant apoplast niche.
Further physico-chemical chartacterisation of infected tissues of resistant and susceptible tomato identified ligno-suberin coatings and tyramine-derived hydroxycinnamic acid amines as the main inducible barriers to bacterial colonisation. In agreement with these findings, overexpression of the ligno-suberin pathway in a susceptible tomato enhanced resistance by restricting *R. solanacearum* movement inside the plant and delaying disease progression.
Finally, activity-based protein profiling of the apoplastic fluid revealed that the p69 family of plant serine proteases is activated upon challenge with *R. solanacearum* in resistant plants and we prove a role of one member of this family in tomato defence to bacterial wilt.
Our findings open new avenues of research to engineer resistance against vascular wilt pathogens.

Plenary Session:

"Plant responses and disease development"

Rooting for resistance: genomic and phenomic analyses reveal new insights into tomato bacterial wilt resistance.

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A major challenge to global crop production is yield loss due to pathogenic microorganisms that cause plant diseases. One of the best means of disease control is genetic plant resistance, but the identification of genes that promote resistance can be difficult, particularly for soilborne diseases. Roots are the first line of defense against soilborne microbes, but root defense responses are not well understood. We use the interaction between tomato and the soilborne bacterium *Ralstonia solanacearum* as a model to understand root immune processes. *Ralstonia* is the causal agent of bacterial wilt and is found throughout the world in hot, humid areas. The pathogen causes devastating disease loss in tomatoes, but few resistance genes have been identified in crops. We used a multi-scale and collaborative approach that has identified factors which promote resistance to *Ralstonia*. Using high-throughput disease phenotyping, we uncovered new genomic regions for resistance. A meta-transcriptomic analysis coupled with root phenotyping revealed that roots of resistant tomato plants can simultaneously grow and activate immune responses against *Ralstonia*, and suggested that water hydraulic mechanisms may function in defense. Together, our results suggest that resistance is a combination of traditional immune pathways as well as molecular and physiological processes that promote cellular homeostasis and root growth.

Invited Conference

O6

SA-independent mechanism in the tomato *diageotropica (dgt)* mutant enhance root-mediated resistance to *Ralstonia solanacearum* K60.

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Bacterial Wilt (BW) is among the most devastating plant diseases in the world. This disease is caused by the soilborne plant pathogen *Ralstonia solanacearum* (*Rs*) and affects more than 200 species of plants. In tomato, resistance to *Rs* is quantitative and the result of many genes and no resistance genes to the US *Rs* strain K60 have been identified in tomato. Transcriptomic analysis of resistant tomato roots showed that at 48 hours post inoculation with *Rs*K60, genes involved in auxin transport and signaling pathways are downregulated. A tomato mutant defective in auxin transport and signaling, known as *diageotropica (dgt)* has enhanced resistance to *Rs*K60. Auxin acts antagonistically with the plant hormone Salicylic Acid (SA), and we have found that *dgt* roots have endogenously higher levels of SA and a strain of *Rs* that can degrade SA is partially virulent on *dgt*. However, after inoculation with *Rs*K60, the expression of SA-dependent response genes is not activated and the SA-deficient double mutant *dgtxNahG* is still resistant to *Rs*K60. In addition, inoculation with *Pseudomonas syringae* pv *tomato* has shown that both *dgt* and its wildtype background are susceptible to this foliar pathogen. Our research suggests that the resistant response to *Rs*K60 of the *dgt* mutant may be due a SA-independent mechanism in roots, and that the *DGT* gene and proper auxin transport and signaling is important for susceptibility to *Rs*K60 in tomato roots. Understanding the role of DGT, auxin, and SA in defense responses to *Rs* in tomato is important for Solanaceae crop improvement.

Travel Award

O7

Virulence of novel *Ralstonia pseudosolanacearum* (phylotype I) isolates from rose, blueberry and mandevilla on seed potato.

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Ralstonia solanacearum (phylotype II), the causal agent of brown rot of potato, causes an estimated US$1 billion in losses each year worldwide, mostly in cooler regions. Our previous work confirmed that an *R. pseudosolanacearum* isolate (phylotype I) acquired from roses can cause severe disease on young Solanaceae plants (other than potato) at 24◦C, with disease incidence reaching 90–100% at 42 dpi. It was not known if this *R. pseudosolanacearum* rose isolate can infect potato under temperate climatic conditions, although in warmer climatic zones *R. pseudosolanacearum* biovar 1 isolates are known to infect outdoor potato crops. Therefore we aimed to determine the aggressiveness of *R. pseudosolanacearum* isolates on *Solanum tuberosum* at different temperatures, as well as to screen for the presence of latent infections in the potato plants and in their daughter tubers. Three recent *R. pseudosolanacearum* isolates were used in this study: PD 7123, P781 and P824, originating from naturally infected rose, mandevilla and blueberry plants, respectively. Three *Solanum tuberosum* cultivars were included in these experiments. Inoculated potato plants were incubated at 20°C or 28°C for 42 dpi. Re-isolation was performed to confirm *R. pseudosolanacearum* as causal agent of the symptoms in the potato plants and in their daughter tubers, as well as to detect *R. pseudosolanacearum* in asymptomatic material. The temperature, the isolate and the potato cultivar used all had a significant effect on disease severity and disease incidence in both the potato plants and their daughter tubers. Despite the absence of symptoms in several cases, re-isolation from symptomless plant/tuber material indicated high frequency of latent infection. The results obtained in this study will be discussed further.

This study was partly financially supported by research grant OS 2016339 project for *R. solanacearum* species complex of the Ministry of Economic Affairs in the Netherlands.

O8

Epidemiology of Blood disease, an emerging threat to banana production.

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Blood disease (*Ralstonia syzygii* subsp. *celebesensis*) causes plant wilt and fruit pulp rot in bananas. The disease occurs in Indonesia and Malaysia, where it leads to significant crop loss. The disease originated on an island in Indonesia, and island borders and quarantine initially slowed its spread. Blood disease recently expanded its geographic range to mainland Malaysia and is now poised to spread unhindered throughout Asia. The disease cycle of *R. syzygii* subsp. *celebesensis* remained largely unexplored due to its past constrained geographic distribution. Therefore, we sought to answer the following questions (i) how does infection occur? (ii) what plant parts are infection courts? and (iii) what are the modes of transmission? Field trials and glasshouse experiments, conducted in Indonesia, with full-grown and potted banana plants were used to demonstrate that *R. syzygii* subsp. *celebesensis* infects through open xylem vessels at male and female parts of banana inflorescences, that sap and ooze from symptomatic plant parts are infectious, and that infection can occur through cut surfaces. Evidence is provided that local dispersal is predominantly through the mechanical transmission of the bacterium by insects, birds, bats, and tools from diseased to healthy banana plants. Long-distance dispersal is most likely through the movement of contaminated planting materials. Our results contribute toward developing improved evidence-based disease management and eradication strategies. Field testing and extension of disease management practices are urgently required to manage the disease and prevent further geographic spread.

Plenary Session:

"Host resistance and crop improvement"

Breeding for potato bacterial wilt (*Ralstonia solanacearum*) resistance in Uruguay.

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Potato bacterial wilt (BW) ranks second among diseases affecting potatoes, worldwide. Some cultivated and wild potato species have been identified earlier as sources of resistance. Instability of resistance, pathogen latency in tubers and restricted agronomic adaptation, prevented adoption of resistant varieties. Development of adapted resistant germplasm, complemented with integrates pest management strategies, would allow more sustainable cultivation of potatoes in affected regions. Since early 2000s, we have evaluated in our program several local accessions of *Solanum commersonii, S. malmeanum* and *S. chacoense* (local wild species), characterized as having high resistance levels to BW. This resistance appears to be quantitatively inherited and expressing transgressive segregation in population studies. Disease resistance screening is performed under controlled conditions using strain UY031 (phylotype IIB, sequevar 1), an aggressive strain isolated in Uruguay from diseased potato. A high sensitivity PCR and culture test was developed to detect latent infections in asymptomatic plants and tubers. Besides, through luminescent and fluorescent transformed bacteria it was possible to determine that in resistant clones the colonization is restricted to roots and a limited number of xylem vessels. Anatomical and metabolic changes were identified in resistant clones at early stages of infection, including repression of the photosynthetic process and proteins related to reactive oxygen species production. Introgression of this resistance to cultivated potatoes has been accomplished via sexual polyploidization using a bridge species (*Phureja*) or directly to *Tuberosum* and several back-crosses (BC) to *Tuberosum* have been obtained. During F1 and BC1 generations, only selection for resistance was applied. From BC2 and further progenies selection was applied for agronomic traits. Promising germplasm at this stage was further evaluated under controlled conditions. Several of these advanced BC3 progenies were also evaluated at naturally infested fields in collaboration with EMBRAPA. Large differences in resistance and other selectable traits, were observed in segregating progenies. Germplasm combining good agronomic performance and high levels of BW resistance up to BC3 was obtained. Nevertheless, most often, latency tests could detect latent infection at asymptomatic plant and tubers. More recently, a characterized local potato wild species core collection was screened, identifying new valuable higher resistance sources. A genome wide association study (GWAS) on a large population of collected accessions is underway to identify genetic architecture of resistance and assisting future resistance selection. Finally, additional germplasm enhancement, trough recurrent selection, complementing adaptation and various resistance sources identified, is being initiated. Most likely, a massive method for BW resistance screening at progeny level should be implemented. Advanced germplasm to obtain should be useful for developing higher resistant germplasm, adaptable trough various BW affected regions.

Invited Conference

Recent advances in breeding potato for resistance to bacterial wilt (*Ralstonia solanacearum*) in Brazil.

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The objective of this pre-breeding activity of the Brazilian potato breeding project was to obtain superior bacterial wilt (BW)-resistant clones free from undesirable traits found on wild species. Genotype selection started at Embrapa in 1987 under the cooperation of CIP-Peru, and CNPq-Brazil. The starting point was a set of genotypes selected for BW resistance by CIP's breeders based on crosses with wild *Solanum* species. In the first decade of the screening tests, we focused on the accessions derived from *S. phureja*, originally selected at the University of Wisconsin, since they better combined resistance and tuber characteristics. Since then, approximately 160,000 clones obtained from true-seeds have been evaluated. Even with *S. phureja* as a genitor, selection index for resistance has been very low, with approximately 1% of selection for reasonable tuber appearance upon artificial and natural inoculations. Even from these, improvement of tuber appearance maintaining high levels of resistance has been difficult due to the complex recombination of alleles, as expected for a tetraploid species. In the first decade, two clones, MB-03 and MB9846-01, were selected for their high and stable resistance upon successive exposures to naturally infested fields with races 1 and 2 of *Ralstonia solanacearum*. They were used in Embrapa's breeding program in recurrent crosses with genitors that favor tropical adaptation, tuber appearance and culinary quality. The most advanced clone so far, MB54-2 (MB9846-01 x BRSIPR Bel), has been used preferentially as a BW-resistant genitor for its high and stable level of resistance as well as high yield, resistance to PVY, good tuber shape and skin, and processing quality. The protocols for BW resistance assessments in the greenhouse and in the field, the levels of resistance achieved, and the characteristic of recently selected clones will be presented.

Experience of using the EFR receptor in potato genotypes to increase resistance to *Ralstonia solanacearum.*

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Bacterial wilt (BW) caused by *Ralstonia solanacearum* is responsible for significant losses in potato (*Solanum tuberosum*) crops worldwide and its incidence in Uruguay has been occasional. Currently, even though it represents a potential risk, prevention measures adopted over the years have kept the disease under control. In our country, race 1 of *R. solanacearum* is considered quarantine and race 3 is regulated, having zero tolerance for seed potatoes production. Resistance genes have been identified in wild species, but their introduction through classical breeding has only achieved partial resistance. The *Arabidopsis thaliana* (At) elongation factor-Tu receptor (EFR) recognizes molecular pattern associated bacterial pathogens to confer antibacterial immunity. The introgression of AtEFR in potato was evaluated in two genetic contexts: a susceptible commercial potato cultivar (INIA Iporá) and an advanced breeding clone (clone 09509.6) with introgressed partial resistance of *S. commersonii*. Evaluations were made in growth chamber, greenhouse and disease-free field. Under controlled conditions, resistance to *R. solanacearum* was evaluated by inoculation of damaged roots. Both INIA Iporá and clone 09509.6 expressing AtEFR showed increased resistance to *R. solanacearum* showing a multiplicative effect of the interaction of the two types of resistance. Using luminescent and fluorescent *R. solanacearum* reporter strains, colonization patterns were compared. There was evidence of delayed and reduced severity of wilt symptoms in AtEFR-transformed genotypes and differential colonization patterns with increased bacterial growth in non-transformed plants. Two events of the INIA cultivar Iporá (Iporá EFR 3 and Iporá EFR 12) were evaluated in the field under pathogen-free conditions compared to the untransformed cultivar. Iporá EFR 12 showed yield stability in the absence of the pathogen, compared to the control. Moreover, as a perspective, the transformed clone 09509.6 produces fertile pollen. Therefore, it can be used in crosses with adapted germplasm to develop local potato varieties with greater resistance in different regions where BW is a problem.

O14

Enhancing the value of our genetic resources: characterization of potato wild relatives from Uruguay for bacterial wilt resistance and other relevant traits for breeding.

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Valuable potato genetic resources can be found in south-eastern South America, such as *Solanum commersonii* (cmm), *S. malmeanum* (mlm) and *S. chacoense* (chc). To efficiently explore, use and conserve these potato wild relatives, we collected 181 accessions representative of their diversity. These accessions were characterized using morphological descriptors and molecular markers, which were analysed together with ploidy level and geographical location. These analyses showed a strong geographical structure, with genetic and morphological groups assigned to each species and clear distribution patterns. We then built a core collection narrowed down to 42 accessions to assess its variability for traits relevant to breeding. This core collection was screened by inoculation under controlled conditions for resistance to bacterial wilt (*Ralstonia solanacearum)* and late blight (*Phytophthora infestans*). Tubers were analysed for total glycoalkaloid content, dry matter content and nutraceutical quality (vitamin C, antioxidants, carotenoids, total protein and mineral contents). We found remarkable phenotypic variability, identifying accessions which combined many favourable traits. More importantly for their further *ex situ* conservation and use, we determined their fertility through assessment of pollen viability, production of unreduced pollen and Endosperm Balance Number (mostly 1EBN for cmm and mlm accessions, and 2EBN for chc accessions). We observed high unreduced pollen production in some 2x and 3x accessions which additionally showed high pollen fertility, making them promising for introgressive hybridization. These results have motivated further studies on the underlying genetic architecture of bacterial wilt resistance in this germplasm and the search for even more extreme sources of resistance with low latent infections. We will also screen this core collection for other important diseases such as common scab and early blight. Our end goal is to preserve and make available a set of accessions with associated information on their value as genetic resources for potato prebreeding.

O15

Identification of molecular markers for bacterial wilt resistance in peanuts.

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Peanut (*Arachis hypogaea* L.), an important source of edible oil and protein, is widely grown in tropical and subtropical regions of the world. Utilization of genetic resistance is the most economic and effective approach to control bacterial wilt in peanut, and identification of quantitative trait locus (QTL) for bacterial wilt resistance (BWR) and linked molecular markers is crucial for peanut breeding. To this end, we systematically evaluated the resistance level of peanut germplasms conserved at CAAS and ICRISAT, upon which linkage mapping and genome-wide association study (GWAS) were conducted to identify QTL for BWR. Linkage mapping of four recombinant inbred lines (RIL) populations identified four major and stable QTLs on chromosomes B02, B03 and A03. The highest effect QTL *qBWRB02-1-1* explained 49.43%-68.86% phenotypic variations across five environments. Through GWAS in a panel consisting of 401 diverse accessions, four significant marker-trait associations (MTAs) were identified in a 1.50 Mb genomic region on chromosome B02, and the resistance allele was derived from the well-known germplasm Xiekangqing with durable resistance to BW. Kompetitive Allele-Specific PCR (KASP) markers were successfully developed for these resistance loci and could be deployed in rapidly selection of resistant lines. These results lay a foundation for the integration of resistance loci from diverse resistant genotypes, which would benefit further genomics-assisted breeding (GAB) to develop novel peanut varieties with enhanced BWR.

O16

Discovery of functional R-genes in resistant blueberry rabbiteye (*Vaccinium ashei*) against *Ralstonia solanacearum.*

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Florida blueberry production is expanding rapidly due to the development of low chill varieties, reaching close to 78 M$ in 2021. In 2016 *Ralstonia solanacearum* was found infecting southern highbush blueberries (*V. corymbosum*) in Florida. This outbreak was not clonal as expected, but three genetically distinct sequevars were identified: Phylotype (Phy) I (sequevar 13), Phy IIA (sequevars 38, and 7). Southern highbush blueberries were susceptible to all sequevars. Since no single control method is completely effective, the solution is developing resistant varieties. To determine if there was resistance in related *Vaccinium* species that could be used in a breeding program, 18 blueberry genotypes including highbush, and rabbiteye (*V. ashei*) species were screened. We found varying degrees of resistance to *Ralstonia* in the rabbiteye group. Our goal is to discover functional resistance genes in *V. ashei* resistant varieties. Our hypothesis is that candidate R-genes can be predicted *in silico* and transcriptomics analyses can be used to discover functional R-genes. Using a reference set of R-gene sequences to build a profile and scan draft genomes of susceptible and resistant genotypes with a Hidden Markov Model-based software (HMMER), 483 and 453 candidate R-genes were predicted, respectively. Additionally, the chromosome-scale and haplotype phased *V. corymbosum* 'Draper' genome was also scanned predicting 1,011 R-genes, confirming that high-quality genomes yield more accurate results. Therefore, we are assembling high-quality genomes of a susceptible highbush and a resistant rabbiteye blueberry with long reads PacBio and Hi-C technologies. Preliminary transcriptome experiments comparing the responses of a resistant rabbiteye, and a susceptible blueberry challenged with the phy I (sequevar 13) strain P824 are currently being analyzed to discover genes differentially expressed. Our findings will be significant for the identification of durable sources of resistance in blueberry that will ultimately lead to the development of crops resistant to bacterial wilt.

Plenary Session:

"Innovative control strategies and integrated management"

Ecology and evolution of phage-bacteria interactions in the rhizosphere: consequences for microbiome functioning and bacterial wilt outbreaks.

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Bacteriophages have been proposed as an alternative to pesticides to kill bacterial pathogens of crops. However, the efficacy of phage biocontrol is variable and this this is mainly because the underlying ecology and evolution phage-bacteria interactions are poorly understood in natural rhizosphere microbiomes[1,2,3]. In this talk, I will present our current work on the role of soil phage communities in constraining the invasions and infections by soil-borne *Ralstonia solanacearum* plant-pathogenic bacterium. Specifically, I will discuss direct and indirect phage effects in complex rhizosphere microbiomes and how pathogen density regulation could be mediated by complex phage-bacteria interactions. From the applied perspective, our findings suggest that phages could be used to engineer the composition of rhizosphere microbiomes by selectively targeting pathogens or other bacteria that interact with the pathogen either positively or negatively. Furthermore, I will highlight how phage selection can rapidly select phage-resistant mutants and how this phage feature could be used as an evolutionary tool to reduce pathogen virulence through costly life-history trade-offs. Together, our findings highlight that soil suppressiveness, which is most often attributed to bacteria, could be determined by the rhizosphere phage communities, highlighting the potential for developing phage therapeutics to control plant pathogenic bacteria in agriculture.

O17

Detection and Analysis of CRISPR Locus in *R. solanacearum* species complex.

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CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) has been widely characterized as a defense system in bacteria and archaea. The *R. solanacearum* species complex (RSSC) is not being left on the sidelines, showing a considerable quantity of their strains possessing the CRISPR array and the CRISPR-associated proteins that confer immunity against various bacteriophages (phages). Recently, lytic phages are being selected and applied to limit RSSC outbreaks and infections with relative success, however, this control alternative could be reduced due to the activation of CRISPR defense in RSSC strains rendering phage therapy ineffective.
In this work, we analyzed 375 genomes of RSSC strains to find the CRISPR locus. We found that 25.5 % of *R. solanacearum* (phylotype II), 14.8 % of *R. pseudosolanacearum* (phylotypes I and III), and 55 % of *R. syzygii* (phylotype IV) strains possess the CRISPR locus. In addition, the RSSC genome sequences were used to identify the respective phages that are restricted by this mechanism of immunity and to detect the respective bacteria-phage relationships. We found 253 different phages that infected different strains of RSSC, by means of the identification of similarities between the protospacers in phages and spacers in bacteria. These phages correspond to 13 phage families/subfamilies mostly from the Caudoviricetes class. Additionally, we found another group of RSSC phages that do not have any vestiges in the bacterial CRISPR system, therefore, they are useful for phage therapy schemes. We build an online database with this information and an analysis tool that helps to select an adequate combination of phages to be used to restrict the growth of RSSC, which accompanies the database. The latter is a fundamental result of this research, allowing for avoiding CRISPR defense when designing phage therapy alternatives to reduce damage caused by RSSC in crops.

Travel Award

O18

Pyramiding host resistance to bacterial wilt and other major soil-borne diseases for integrated management in peanut.

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Bacterial wilt (BW) caused by *Ralstonia solanacearum* is the only important bacterial disease in peanut (*Arachis hypogaea*), but in all the BW-infested regions peanut is also heavily affected by several other soil-borne fungal diseases including pod rot, stem rot, scab, and even *Aspergillus* infection. Both bacterial and fungal soil-borne diseases have been proven difficult to control and thus utilization of host-plant resistance is the most prioritized approach in management. Worldwide, more than 150 accessions of cultivated peanut and its related wild *Arachis* species with wide diversity have been identified as highly resistant to BW. Most of the BW-resistant peanut lines have been also evaluated for their resistance to various soil-borne fungal diseases, from which, elite germplasm lines have been identified as resistant to pod rot, scab and aflatoxin production besides resistance to BW. More recently, resistance to *Aspergillus* infection resistance of peanut shell has also been transferred into BW-resistant genotypes. Up to date, the progress of combining resistances to major soil-borne diseases have effectively facilitated the integrated management of these important diseases and hence promoted the production increase of peanut in the BW-infested regions.

O19

Bioassay-based method for screening biological control agents against tobacco bacterial wilt.

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Tobacco bacterial wilt (TBW) caused by *Ralstonia pseudosolanacearum* is one of the most important diseases in the tobacco production. It reduced tobacco yield severely and caused serious economic losses in some areas of Guizhou, Fujian and Yunnan provinces in recent years. Effective methods are urgently needed to fight against this disease. Biological control received more attention for its safety to the environment, human being and animal. Here, we developed a tobacco bioassay-based method for effective screening of biological agents against TBW. A relatively oligotrophic medium (0.1% Casamino acid, 0.1% nutrient broth and 1.5% agar) was used for isolating soil bacteria. Instead of antagonistic screening on plate, an *in vivo* bioassay was developed for preliminary screening biological agents. Seeds of susceptible tobacco cultivar "Honghuadajingyuan" were sowed in 112-well tray. The seedling roots were injured by cutting 4-5 weeks later and then pre-inoculated with bacterial suspensions (10^8 CFU/mL). Medium broth was treated as the control. Two days later, seedlings were inoculated with *R. solanacearum* (10^8 CFU/mL), and the disease severity was assessed at about 20 dpi when the symptoms were fully developed in the control. By using our bioassay screening, totally 795 strains against TBW were selected out from approximately 6720 soil bacterial isolates. Suppressive capacities of obtained bacteria were further confirmed by using 8 seedling plants treated as mentioned above. Furthermore, 174 and 29 isolates were used for first round and second round pot experiments in greenhouse. Finally, 8 isolates with the control efficiency better than 30% were obtained. Based on the 16S rRNA gene sequence similarities, 8 strains were most closely related to *Enterobacter tabaci*, *Aeromonas hydrophila*, *Ralstonia mannitolilytica*, *Stenotrophomonas indicatrix*, *Pseudomonas nitritireducens*, *Bacillus subtilis*, *Enterobacter mori* and *Stenotrophomonas* sp., respectively. Interestingly, all isolates showed disease-suppressive capacities in bioassay test did not inhibit growth of *R. solanacearum* on plate, indicating that other mechanisms could be involved to control TBW. Above results suggest that our screening combined of poor nutrient medium and tobacco bioassay is an effective approach to obtain potential biological agents which differ from those obtained by using antagonistic test-based method.

O20

Integrated strategies to manage bacterial wilt (*Ralstonia*) of tomato in North Carolina, USA.

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Bacterial wilt caused by *Ralstonia solanacearum*, was first documented in North Carolina (NC) tobacco fields in 1903. The pathogen is a persistent problem from the subtropical to temperate tomato production regions of NC and has been consistently characterized as race 1, biovar 1, phylotype II, sequevar 7 strains. Growers experience plant loss and in severe cases abandon land to tomato production due to lack of efficacious control. Field experiments and grower experience documented lack of control using soil fumigation (with chloropicrin as the main active ingredient). Commercial or experimental hybrids frequently had significantly less plant death compared to susceptible controls but have not proven commercially viable. Multiple years of on-farm research identified rootstocks that confer high levels of bacterial wilt resistance (up to 100%) compared to susceptible controls that suffer plant death losses (up to 100%). Diverse cultivars as scions were included on an array of rootstocks with no incompatibility issues identified. New businesses expanded the capacity to graft plants in NC and other regions of the USA and complemented with extension-based recommendations of resistant rootstocks, has led to adoption of grafted plants across the state. However, the extra cost of grafted plants has limited adoption in tomato growing systems that cannot bear the risk of the extra expense. Growers and emergent research projects are also experimenting with modifying the pH of soils (to high pH levels) and exploring anaerobic soil disinfestation plus the microbial ecology of soils, respectively, as additional tactics to manage bacterial wilt. For over 120 years, bacterial wilt has been a problem in NC and the surrounding southern States. The progressive integration of soil treatments or amendments, host resistance, and advanced understanding of the biology and ecology of the pathogen and beneficial microbes are important components in developing integrated strategies to manage this persistent pathogen.

Travel Award

Plenary Session:

"Plant-pathogen interactions within the phytobiome"

Bacterial wilt resistance and root microbiome in tomato.

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Plant-associated microbiota plays an important role to modulate plant function. To unveil the role of plant microbiome in plant disease resistance, we used the tomato plant and bacterial wilt (BW) as a model system. Tomato cultivars Hawaii7996 (H7996) as a BW resistant cultivar and Moneymaker (MM) as a susceptible cultivar were used for our microbiota and metagenome studies. Analysis of both microbiota and metagenome in the rhizosphere of two tomato cultivars, with a subsequent transplant experiment, indicated that rhizosphere microbiota of the resistant plant contributes to the BW resistance in tomato plant. Both metagenome binning and microbial genome information revealed a member of Flavobacteria which is highly enriched in the rhizosphere of H7996. Using the genome information, we isolated a novel bacterium from the rhizosphere of H7996. The novel bacterium suppressed BW progress in a susceptible cultivar. This result revealed a pivotal role for native microbiota in protecting plants from infection. Further, we established a system to study plant-microbiome interaction under defined soil condition with various microbiota transplants. A tomato cultivar H7996 grown under the defined soil with microbiota transplant exhibited various degree of microbiota-dependent BW resistance. Comparison of rhizosphere microbiotas in H7996 and MM with two different microbiotas transplant showed the distinct microbiota structures and differential bacterial abundance and composition. Based on the microbiota transplant and microbiotas analyses, we constructed synthetic communities (SynCom) and investigated the effect of SynCom treatment in tomato rhizosphere for the modulation of BW resistance in H7996. A specific SynCom confers H7996 plant with cultivar-specific protection against BW. The microbiota transplant and SynCom approach suggested that cultivar-selective enrichment of rhizosphere microbes plays a pivotal role in protecting plants against BW and a specific microbiota modulates the quantitative resistance of tomato against BW.

Potential role of rhizosphere modulation in controlling *Ralstonia solanacearum* caused bacterial wilt.

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Ralstonia solanacearum biovar2-race3 (*Rs* r3b2) is an epidemic soil-borne bacterial phytopathogen causing brown rot disease in potato plants and is endemic in many areas of the world. We have recently reported that the proportion and diversity of *in vitro* antagonists towards *Rs* isolated from bulk soil and different spheres of potato plants grown under field conditions in three different soil types was mainly shaped by the plant sphere and to a lesser extent by the soil type. The proportion of antagonists was higher in the endophytic compartments and the vast majority of antagonists belonged to *Pseudomonas* and produced siderophores. Two bacterial isolates from potato tuber endosphere with *in vitro* antagonistic activity toward *R. solanacearum* (B3B), *Bacillus velezensis* (B63) and *Pseudomonas fluorescens* (P142) were tested for rhizosphere competence and efficient reduction of B3B abundance and wilt symptoms on greenhouse-grown tomato. Furthermore, we investigated how the pathogen and/or the antagonists altered the composition of the microbiome (bacteria/archaea) composition based on the sequence analysis of 16S rRNA gene fragments amplified from total community DNA. Plants inoculated with B63 or P142 showed a drastically lower abundance of B3B in the rhizosphere and significantly reduced wilt disease symptoms compared to the non-inoculated pathogen control. Pronounced shifts in microbiome composition were observed in response to the inoculation of B63 or P142 in the presence or absence of the pathogen B3B and numerous dynamic taxa were identified. Although competitive niche exclusion cannot be excluded, it is more likely that the inoculation of P142 or B63 and the corresponding microbiome shifts primed the plant defence against the pathogen B3B. While both inoculants are promising for microbiome assisted control of *Rs* under field conditions, more simple agronomic measures (adding organic matter) might stimulate growth and activity of indigenous potential plant beneficial bacteria such as *Pseudomonas* through microbiome modulation and thus stimulate indigenous soil suppressiveness towards *Rs*.

O21

Transcriptional landscape of the *Ralstonia solanacearum* life cycle: identification of key genes for growth in soil

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Transcriptomic studies can provide a detailed understanding of the bacterial responses to environmental cues. To date, many studies in phytopathogenic bacteria have only focused on the *in planta* infection stages and the environmental stages of their life cycle have been overlooked. *Ralstonia solanacearum* has a complex life cycle, as it can survive in the soil environment for years before infecting its host. The soil is thus a crucial niche for disease establishment and a source of contamination and disease outbreaks. To date, no studies have focused on *Ralstonia solanacearum* gene expression in the environment.

Here we describe transcriptome of *R. solanacearum* UY031 when the bacterium thrives in a natural soil. We compared the results to our previously published transcriptomes obtained *in planta* or *in vitro* and identified a set of genes that are specifically expressed in the soil. Remarkably, most of these genes were associated with stress responses. Functional characterisation of several soil-induced stress genes reveals that antioxidant activities are required for the bacterium to survive in soil. This is first study describing *R. solanacearum* gene expression during the unstudied soil stage, and we identify a set of genes potentially crucial for survival in this environment. Increasing our understanding of the *R. solanacerum* life cycle will help to develop novel targets for disease control.

O22

Exposing phage to multiple host genotypes can improve outcome of phage training with phytopathogenic *Ralstonia solanacearum* bacterium

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The "Phage Training" is a process of increasing phage infectivity by pre-adapting them to their bacterial hosts using experimental evolution. Here we used phage training to pre-adapt one phage against three genotypes of *Ralstonia solanacearum* phytopathogenic bacterium either in pairwise or multi-bacterial co-cultures. We hypothesised that we would see an increase in phage infectivity and a potential delay in phage resistance. With phages exposed to multiple pathogen genotypes exerting more generalist adaptations relative to specialist adaptations rising in pairwise cultures. We found that phages trained against multiple *R. solanacearum* genotypes had an advantage over phages trained against single bacterial genotypes in terms of supressing ancestral bacteria growth. Interestingly, in some cases, we found that phage training provided little to no improvement with the ancestral phage outperforming the trained phages. Furthermore, the outcome of phage training was highly dependent on the *R. solanacearum* genotype, potentially due to difference in phage defence system repertoire. Moreover, even though phage training led to improved suppression of *R. solanacearum* growth, it could easily evolve resistance against trained phages. Whether phages can engage in coevolutionary arms race with the pathogen will be discussed based on the results of ongoing long-term coevolution experiment. Together, our findings suggest that using phage training to improve phage infectivity might not be as straightforward with *R. solanacearum*, potentially due to pre-existing history with phages in agricultural and natural environments.

O23

Indirect reduction of *Ralstonia solanacearum* via pathogen helper inhibition.

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The rhizosphere microbiome forms a first line of defense against soilborne pathogens. To date, most microbiome enhancement strategies have relied on bioaugmentation with antagonistic microorganisms that directly inhibit pathogens. Previous studies have shown that some root-associated bacteria are able to facilitate pathogen growth. We therefore hypothesized that inhibiting such pathogen helpers may help reduce pathogen densities. We examined tripartite interactions between a model pathogen, *Ralstonia solanacearum*, two model helper strains and a collection of 46 bacterial isolates recovered from the tomato rhizosphere. This system allowed us to examine the importance of direct (effects of rhizobacteria on pathogen growth) and indirect (effects of rhizobacteria on helper growth) pathways affecting pathogen growth. We found that the interaction between rhizosphere isolates and the helper strains was the major determinant of pathogen suppression both *in vitro* and *in vivo*. We therefore propose that controlling microbiome composition to prevent the growth of pathogen helpers may become part of sustainable strategies for pathogen control.

Hayward-Prior Award Session

O9

Genomic and phenotypic diversity of *Ralstonia pseudosolanacearum* infecting multiple hosts in Cambodia

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Thousands of heterogenous strains in the *Ralstonia solanacearum* Species Complex (RSSC) cause bacterial wilt disease on a broad host range worldwide. This distribution and diversity mean that management strategies and resistance breeding must be adapted to local RSSC strains. Bacterial wilt has long been present in Cambodia, but little is known about the diversity and distribution of RSSC strains in Cambodia. We isolated Cambodian RSSC strains from symptomatic hosts including tomato, long bean, bitter gourd, and hot pepper in three provinces throughout the country. Isolates were sub-classified into phylotype and sequevar using the phylotype multiplex PCR and partial endoglucanase gene sequencing, respectively, and results were confirmed using genomic data. All isolates belonged to phylotype I, also known as *R. pseudosolanacearum*. Additionally, the isolates clustered into three distinct sequevar groups. To better understand how the Cambodian isolates fit into the broader RSSC, we sequenced the genomes of the Cambodian isolates with the Illumina NextSeq 2000 and conducted comparative genomic analysis. Whole genome rather than solely endoglucanase gene analysis results revealed that Cambodia harbors extensive bacterial wilt pathogen diversity, even in a single field. Ongoing phenotypic diversity assays with the Cambodian *R. pseudosolanacearum* isolates will be aligned with genotypic data. These new insights on pathogen diversity can ensure appropriate screening for wilt-resistant vegetable varieties for Southeast Asian growers.

Travel Award

Travel Award

O10

Multiple and overlapping chemoreceptors within *Ralstonia* species have diverging ligand specificities.

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Ralstonia solanacearum exploits motility and chemotaxis (directional movement along a chemical gradient) to locate host roots. Membrane-bound chemoreceptors drive chemotaxis and contain a conserved signal transduction domain and highly sequence divergent ligand binding domains (LBDs). Genetically diverse members of the *Ralstonia solanacearum* species complex (RSSC) have 17+ structurally diverse chemoreceptors encoded in their genomes. We hypothesize that variability in the chemotactic preferences across the RSSC plays a role in locating preferred host plants. Here we probe the chemotactic range of *Ralstonia* by generating chimeras by fusing LBDs of the *Ralstonia* chemoreceptors with the transduction domain of a two-component system that controls gene transcription in *E. coli*. Using reporter strains designed to measure transcriptional readout, we characterized the ligand ranges of LBDs from *Ralstonia* model strains predicted as amino acid sensors. RSSC genomes contain two paralogous amino acid sensors, McpA and Rsc3307. Interestingly, despite high sequence similarity between the Rsc3307 orthologs, the amino acid sensors tested from *Ralstonia solanacearum* IBSBF1503 (IIB-4) and *Ralstonia syzigii* PSI07 (IV-10) had variable ligand ranges. *Ralstonia* mutants lacking *mcpA* or RSc3307 were confirmed to have defects in sensing amino acids. Further, we show that these two receptors sense different classes of amino acids and are not fully redundant in their physiological role. *In planta*, the lack of these two receptors has a minor effect on *Ralstonia* ability to infect plant hosts. Our data indicate that subtle changes in LBD sequence can have significant influences on which chemicals a bacterium can detect. Variability in the chemical repertoire of a *Ralstonia* strain could affect the efficiency a host plants' roots are identified. However, the chemotaxis of *Ralstonia* is complex and contains redundancies. Our chimeric approach to decoding the chemical-sensing capabilities allows us to determine individual ligand-receptor interactions to identify influential signals within the abundance of attractive plant signals.

O11

Regulation of the micacocidin production-related gene *RSc1806* and its involvement in virulence of *Ralstonia pseudosolanacearum* strain OE1-1

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Iron is an essential element for most organisms, including bacteria. Most bacteria secrete powerful ferric iron–chelating molecules called siderophores to scavenge iron from their environment. The iron depletion leads to an induction of siderophore production in most Gram-negative bacteria. The Gram-negative β-proteobacterium *Ralstonia solanacearum* species complex (RSSC) produces staphyloferrin B and micacocidin as siderophores. The LysR family transcriptional regulator PhcA activated during quorum sensing (QS) regulates QS-dependent genes responsible for QS-regulated phenotypes including production of staphyloferrin B. The *RSp0424*-deletion mutant loses staphyloferrin B productivity but retains virulence. The *RSc1806*-deletion leads to a loss in micacocidin productivity. However, we have little information on the regulation mechanism of micacocidin production. To analyze the regulation of micacocidin production-related genes (Mic-genes) including *RSc1806* in a phylotype I strain of RSSC, *R. pseudosolanacearum* strain OE1-1, we performed the transcriptome analysis using RNA-seq in the strain OE1-1. The transcript levels of both Mic-genes including *RSc1806* and staphyloferrin B production-related genes (SB-genes) including *RSp0424* in the strain OE1-1 incubated in the medium containing $FeSO_4$ at concentration of 450 nM (Fe-medium) exhibited significantly lower accumulation, compared with those in the medium without $FeSO_4$ (nonFe-medium). QS activity of the strain OE1-1 incubated in the nonFe-medium was significantly reduced, compared to that in the Fe-medium. When incubated in the nonFe-medium, QS-deficiency led to a significantly enhanced expression level of SB-genes but not Mic-genes. The *RSc1806*-deletion led to a significantly reduced QS activity in incubation with or without $FeSO_4$. Furthermore, the *RSc1806*-deletion led to a loss in virulence. Taken our results together, iron depletion in OE1-1 cells induces micacocidin production, which may influence QS activity of the strain OE1-1 independently of iron intracellular concentration and be involved in its virulence.

Travel Award

Travel Award

O12

Effect of bacterial wilt incidence on growth and yield response of tomatoes, potatoes and capsicum under various field soil amendments.

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Bacterial wilt caused by *Ralstonia solanacearum* is reported to be one of the major challenges affecting solanaceous crops farmers in Kenya. The main objective of this study was to establish the effect of bacterial wilt incidence on growth and yield response of Tomatoes, Potatoes and Capsicum under various field soil amendments. The study was laid out as randomized complete block design (RCBD) in split plot arrangement for two seasons in the field. The experiment was conducted at the experimental plots at KARLO- NARL, Kabete Nairobi County between July, 2017 - September, 2017 and between November, 2017- January, 2018. The three choice crops were inoculated with prepared pure bacterial isolates: 18 (2T-Kiambu-Low Land), 71(2A-Nyeri-Low Land), 67 (2A-Nyeri-High Land), 83 (2T-Kirinyaga-Highland) and MX (18/71/67/83). A plot measuring 66 m by 28.5 m was marked, cleared, ploughed, harrowed and demarcated into 150 plots each measuring 2.4 m x 3.75 m. Spacing of the host crops of interest: potato - (Tigoni variety), tomato (Caj variety) and capsicum (Califonia Wonder) was carried out at 75 cm between the rows and 30 cm within the rows. The treatments were Chalim™, Super-hydro-grow polymer + Metham sodium, Metham sodium, Metham sodium + Orange peel, Super-hydro-grow polymer, Brassica tissues, Chalim™ + Super-hydro-grow polymer, Brassica tissue + Orange peel, Metham sodium + Super-hydro-grow polymer and Control (no amendments). Assessment of the bacterial wilt incidence started at the onset of wilt symptoms. Growth and yield parameters; total crops, total flowering height, average flowering, total fruits, total weight of fruits per crop, average number of fruits per crop, average weight of the fruit, fresh weight of shoot, fresh weight of root, dry weight of root and dry weight of shoot were taken. Brassica tissue + Super-hydro-grow polymer, Brassica tissue + Orange peel and Brassica tissue were superior in maintaining high yields and growth parameters in capsicum, tomatoes and potatoes. Application of different soil amendments had significant effect on incidences of bacterial wilt on potatoes, tomatoes and capsicum at $P< 0.05$ level of significance except for Metham sodium + Orange peel soil amendment which was not significantly different from control in capsicum. The study established a negative correlation between bacterial wilt incidences on capsicum, potatoes and tomatoes on all the growth parameters and yields. We recommend farmers to use Brassica tissue + Super-hydro-grow polymer or Brassica tissue + Orange peel in controlling bacterial wilt and enhancing growth parameters and yields in most solanaceous crops.

Travel Award

O13

Biological and molecular characterization of bacteriophages with biocontrol potential against bacterial wilt caused by *Ralstonia solanacearum* in tomato crops.

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Bacterial wilt disease, caused by *Ralstonia solanacearum* (*Rs*), is one of the major diseases of tomato and other solanaceous plant. Currently, the phylotype IIB Sequevar 1, it has been reported in potatoes and tomato crops and classified as quarantine pests in Chile. Copper-based agrochemicals and antibiotics are the most frequent alternative to manage this disease, however, these methods produce phytotoxicity and environmental contamination. Therefore, desirable to develop alternative strategies to manage *Rs*. Biological methods were reported as an innovative approach to prevent bacterial infection, especially the use of bacteriophage (phage), a virus that can exclusively attack the bacterium host. Analyzing soil samples from tomato crops in the Valparaíso region, we isolated 15 phages using the double agar overlay method. Enzymatic digestion patterns with *MseI* endonuclease revealed a genomic differentiation between the 15 phages. Hence, we selected only six for biological and molecular characterization. Electron micrographs obtained until the moment showed the morphology of four phages belonging to *Podoviridae*, and one is the *Myoviridae* family. The six phages showed a large host range against 12 strains of *Rs* isolated from tomato and potato crops in different geographic zones of Chile. More interestingly, using different proportions of phages to infect a culture of *Rs* to analyze lytic activities, we observed all phages inhibit the growth of the host cells compared with the culture of *Rs* without phages. Finally, phages were stable in incubation at pH 7, 9, and 11 in SM buffer solution for 1 hour. In contrast, we observed a significant decrease in the phage titers when phages were at pH 2 and 5 in SM solution. In conclusion, in the present work, we detected and isolated phages from environmental samples from tomato crops in Chile that have lytic activity against *Rs in vitro* conditions.

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Abstracts of Poster Presentations

Mon 20 — POSTER SESSION A

Code	Name of presenter author	Poster title
A1	Jane Ray	Current phylogenetic status of the *Ralstonia solanacearum* species complex in Australia
A2	Martina Stoycheva	*Ralstonia solanacearum* species from different continents show clear differences in their evolvability and genetic variation
A3	Caitilyn Allen	*Ralstonia solanacearum* cool virulence on potato is quantitative and evolved repeatedly
A4	Tiffany Lowe-Power (Nathalie Aoun)	Investigating the adaptive mechanisms of *Ralstonia solanacearum* species complex host range
A5	Maria Bergsma-Vlami	*Ralstonia pseudosolanacearum* (phylotype I) in waterways and bittersweet (*Solanum dulcamara*) in the Netherlands.
A6	Luciellen da Costa Ferreira	Prospecting interspecific pathogenic and growth characteristics on some isolates of *Ralstonia solanacearum*
A7	Antinéa Sallen	Evaluation of the phenotypic and genotypic diversity of *Ralstonia solanacearum* in metropolitan France and the risks for emergence of other species of the *Ralstonia* spp. complex
A8	Xiao-man She	Genomic sequencing of different sequevars of *Ralstonia pseudosolanacearum* strains isolated sunflower
A9	Nicole Vasconez	The bacterial wilt of tomato caused by *Ralstonia solanacearum*: an emerging disease in Chile
A10	Liying Yan	Genetic diversity and pathogenicity variation of *Ralstonia solanacearum* strains from peanut in central and southern China
A11	Maka Muradashvili	Result of whole genome sequence-based characterization of eight *Ralstonia solanacearum* isolated in Georgia
A12	Marie Veronique Nomenjanahary	Genetic diversity of the type III effector RipAX2 in the *Ralstonia so)lanacearum* species complex and its impact on the deployment of eggplants carrying the EBWR9 resistance locus in the South-West Indian Ocean
A13	Kristi Kabyashree	*Ralstonia solanacearum* preferential colonization in the shoot apical meristem explains its pathogenicity pattern in tomato seedlings
A14	Shili Li	Identification of the RSc1155 gene involved in cinnamic acid chemotaxis in plant infection by *Ralstonia solanacearum*
A15	Elena Orellano	Participation of *Ralstonia solanacearum* catalases in the plant-pathogen interaction
A16	Virginia Ferreira	Calcium increases bacterial wilt resistance in potato and decreases *Ralstonia solanacearum* virulence.
A17	Marcela González	Evaluation of sources of resistance against *Ralstonia solanacearum* and *Clavibacter michiganensis* in tomato
A18	Huaiyong Luo (Li Huang)	Improving nitrogen fixation capacity of bacterial wilt-resistant peanut genotypes by discovering and integrating dominate genes for nodulation
A19	Qipeng Jiang	Soil properties drives bacterial community assembly in tobacco rhizosphere affecting bacterial wilt disease
A20	Huifang Jiang	Discovery of a Novel QTL on chromosome B03 for resistance to bacterial wilt in peanut variety ICG12625
A21	Valentina Stancov	Screening a core collection of potato wild relatives from Uruguay for bacterial wilt resistance
A22	Luciana Viera	Evaluation of resistance to bacterial wilt in advanced potato (*Solanum tuberosum* L.) germplasm
A23	Nicolás Núñez	Optimizing screenings to simplify phenotyping and dissect the genetic architecture of bacterial wilt resistance in potato wild relatives from Uruguay using GWAS

Tue 21

POSTER SESSION B

Code	Name of presenter author	Poster title
B1	Sara Franco Ortega	Reservoir hosts of *Ralstonia solanacearum*: a key element of the fight against bacterial wilt
B2	Alba Moreno Pérez	Single-cell RNA-seq strategy to identify effector-targeted plant cells
B3	Maria Bergsma-Vlami	Virulence assessment of *Ralstonia solanacearum* (phylotype II) isolated from ornamental *Rosa* spp. plants
B4	Myriam Izarra	Relative expression of βhpmeh gene in transgenic events of the potato variety 'Desiree' related to resistance to bacterial wilt caused by *Ralstonia solanacearum*
B5	Bayo Siregar	Bacterial wilt disease of *Eucalyptus pellita* in Indonesia: disease trigger factors, pathogen and host plants diversity
B6	Adan Alvarado Ramirez	Microbial community physiological profiles and isolation of *Ralstonia solancearum* biocontrol agents of field-growing tomato plants
B7	Belén Álvarez	Effect of conservation by lyophilization on survival and in planta biological control of lytic bacteriophages of *Ralstonia solanacearum*
B8	Belén Álvarez	Characterization of *Solanum lycopersicum* defense responses to biocontrol with lytic *Ralstonia solanacearum* bacteriophages
B9	Belén Álvarez	Genomic and phylogenetic characterization of *Ralstonia solanacearum* bacteriophages useful for biocontrol in irrigation water and in plant
B10	Wei Ding	Green control technology and products innovation of new strategies based on biological barrier against tobacco bacterial wilt disease
B11	Qi Huang	Isolation and characterization of a jumbo *Ralstonia*-infecting phage with promising biocontrol potential
B12	Gao-Fei Jiang	Wilt disease suppression via rhizosphere microbiome transplant
B13	Gao-Fei Jiang (Tianjie Yang)	Enhancement of synbiotics on microbial resistance against soil-borne *Ralstonia* disease
B14	Elena Orellano	*Gluconacetobacter diazotrophicus* promotes resistance to *Ralstonia psuedosolanacearum* inducing plant defense routes
B15	Mauricio Rossato	Reaction of chickpea cultivars to bacterial wilt, a new disease to a crop under expansion in Brazil
B16	Mauricio Rossato	Grafting onto "baquicha" confers super protection on tomato against bacterial wilt
B17	Liang Yang	Sustainable natural bioresources: coumarins mediate resistance against *Ralstonia solanacearum* in tobacco through jasmonic acid signaling
B18	Lilia Carvalhais	Novel assay to detect *Ralstonia solanacearum* causing Moko disease in banana
B19	Amandine Cunty	Missions of the Plant Health Laboratory as a National Reference Laboratory regarding the detection of *Ralstonia solanacearum* species complex
B20	Luis Otavio Saggion Beriam	Molecular differentiation of *Ralstonia solanacearum* biovars I, II and III
B21	Luis Otavio Saggion Beriam	First report of *Ralstonia solanacearum* on *Kalanchoe blossfeldiana* in Brazil
B22	Hyoung Lee	Microbiome and transcriptome analysis of a bacterial wilt resistant tomato plant transplanted with two different soil microbiotas
B23	Maka Muradashvili	Phage therapy for biocontrol of bacterial wilt in strategic crops

A1

Current phylogenetic status of the *Ralstonia solanacearum* species complex in Australia

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The *Ralstonia solanacearum* species complex (RSSC) comprises a large group of strains that vary in host range and biology and is one of the most destructive plant pathogenic bacterium worldwide. The complex causes wilt in over 200 plant species. Over the decades, the *R. solanacearum* complex were classified in different ways confusing the identity of strains and disease causal agents. This diverse group has been resolved into a stable taxonomic framework of three species and subspecies (Safni et al. 2014). Phylogenetic relationships within-species or strain identity are determined using a single marker gene-based sequevar system and a whole genome-based LINs system (Sharma et al. 2022; Fegan and Prior 2005). Australia cannot adequately evaluate the risk of exotic *Ralstonia* strains because the identity of endemic strains in terms of the modern taxonomic framework is unknown. Therefore, the overall objective of this project is to determine the identity and distribution of *Ralstonia* strains in Australia. Thus far, over 175 RSSC isolates have been retrieved from Australia's culture collections, isolates collected through passive and active surveillance programs. Australia's *Ralstonia* collections date back to the 1960's and originate from a diverse range of host genera including *Solanum*, *Capsicum*, *Strelitzia*, *Heliconia*, *Zingiber*, *Vaccinium*, *Lactuca*, *Olea*, *Nicotiana*, *Annona*, *Zinnia*, *Galphimia*, *Acacia*, *Cucurbita*, *Eucalyptus*, *Salvia*, *Sorghum*, *Synedrella*, *Diospyros*, *Archontophoenix*, and *Dahlia*. This project will create a baseline of the RSSC strains present in Australia.

A2

Ralstonia solanacearum species from different continents show clear differences in their evolvability and genetic variation.

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Abstract: Bacterial strains in *Ralstonia solanacearum* species complex (RSSC) pose global risk for food production, due to their ability to infect several crops. While this is partly explained by its rapid adaptation to different climates and plant hosts, *R. solanacearum* genetic diversity in agricultural systems and environmental reservoirs is poorly understood. I will present a population genomic analysis of RSSC, exploring the evolution of this pathogen at two levels: within whole country (UK) and within and between tomato fields in China. At the country level, we analysed 170 UK isolates, spanning 26 years of sampling including all UK outbreaks on record since the first one in 1992. Time series analysis shows that the UK population of phylotype IIB-1 has remained highly clonal after the introduction into the wild weed *S. dulcamara* host with detectable founder effect and loss of accessory genes during the initial colonisation. On the spatial scale only a small signal of neutral variation driven by insertion sequence movement could be detected. On the within country level, we genotyped and phenotyped 96 isolates from 4 tomato fields in China, where *Ralstonia pseudosolanacearum* is prevalent species. We observed notable genetic and phenotypic diversity within agricultural monocultures, and within all fields, isolates belonging to two genetically distinct lineages coexisted and differed regarding the presence of virulence factors, T3Es, competitiveness, and biofilm production. Supposedly the coexisting lineages possess different adaptive strategies, enabling two 'ecotypes' to co-occur in a tomato's rhizosphere due to niche sharing. Together these results show that certain RSCC strains can remain clonal and unchanged for decades in natural reservoirs, while others show high standing variation even within agricultural monocultures. Linking RSSC genetic and phenotypic variation with its disease epidemiology is important for understanding the ecology and evolution of this species and developing new control strategies targeting different pathogen genotypes.

A3

Ralstonia solanacearum cool virulence on potato is quantitative and evolved repeatedly.

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Potato brown rot (BR) is an increasingly important cause of crop losses. Some *Ralstonia solanacearum* strains cause BR in cool tropical highlands and occasionally in temperate zones. To reduce spread, these strains are subject to strict quarantines. They are known for regulatory purposes as Race 3 biovar 2 (R3bv2) but no phenotypic test identifies them. It is unknown if all cool virulent strains belong to R3bv2 or if all R3bv2 are cool virulent. To improve regulation of threatening *R. solanacearum* strains independently of the R3bv2 designation, we used genomes of >200 *R. solanacearum* phylotype IIB strains to build a strong phylogenetic classification and identification framework. This reinforced that a single *R. solanacearum* IIB-1 clonal lineage of South American origin is responsible for the destructive global potato BR pandemic. This BR pandemic lineage can be identified by whole genome sequencing and has a unique Life Identification Number (LIN) in the public LINbase database. We measured virulence and host colonization levels of representative strains on tomato and potato plants at 22°C and 28°C. These assays revealed that cool virulence is quantitative and that tomato is not a reliable proxy for potato. Further, cool virulence and host preference are interacting traits. Finally, we correlated cool virulence phenotypes with the phylogeny-based strain classification. Some South American IIB-1 strains that are currently regulated as R3bv2 are not cool virulent. Based on these results, we propose that quarantine regulations be restricted to the phenotypically and genotypically distinct BR pandemic lineage, and that in the future, *R. solanacearum* strains be identified using whole-genome sequences or molecular markers unique to that lineage. Concerningly, several lowland tropical *R. solanacearum* strains in the IIB-4 subgroup wilt potato plants, cause latent infections, and colonize tubers at 22°C similarly to strains in the brown rot pandemic lineage.

A4

Investigating the adaptive mechanisms of *Ralstonia solanacearum* species complex host range.

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Pathogen host range is a key trait that affects epidemiology and pathology. Generalist pathogens have evolved adaptive mechanisms that create high genetic diversity. This genetic diversity allows pathogen populations to cross species barriers and infect different host species. However, adaptive mechanisms of generalist pathogens that co-evolve with plant species remain poorly understood. My project aims to dissect *Ralstonia's* adaptations that affect host physiology and defenses in *Ralstonia solanacearum* species complex. To identify *Ralstonia's* adaptation genes, I use two advanced genomics approaches that are based on forward genetic screening (RB-TnSeq) and genome-wide association (GWA) mapping. My pilot high-throughput genetic screens revealed the presence of common and host-specific genes that promote or hinder *Ralstonia* growth under varying levels of plant defenses. Many of these genes have diverse or unknown functions. However, my screens show that several type-3 effectors specifically impede *Ralstonia*'s growth in specific tomato varieties. Moreover, my preliminary microbial genome-wide association mapping analysis on 25 *Ralstonia solanacearum* IIB-4 strains revealed the potential involvement of the *pksM* gene in *Ralstonia* colonization. This project sheds light on novel bacterial adaptation mechanisms to host range.

A5

Ralstonia pseudosolanacearum (phylotype I) in waterways and bittersweet (*Solanum dulcamara*) in the Netherlands.

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Ralstonia solanacearum (phylotype II), the causal agent of the brown rot bacterium of potato, is a major threat to the potato industry. As it has the potential to survive in surface water, annual surveys in waterways are performed to monitor the spread of *R. solanacearum* in the Netherlands. In autumn 2020, the outcome of a diagnostic investigation on water samples originating from surface water in two provinces in the Netherlands confirmed the presence of *R. pseudosolanacearum* (phylotype I). *R. pseudosolanacearum* (phylotype I and III) is one of the three described bacterial species inside the *Ralstonia solanacearum* species complex (RSSC), next to *Ralstonia solanacearum* (phylotype II) and *Ralstonia syzygii* (phylotype IV). Additional surface water samples taken in 2021 within a 5 km radius of the initial findings in 2020 confirmed the presence of *R. pseudosolanacearum* (phylotype I). Further, analysis of lower stem and root parts of bittersweet (*Solanum dulcamara*) collected from the same areas demonstrated the systemic presence of *R. pseudosolanacearum* in this plant. This suggests that *R. pseudosolanacearum* (phylotype I) overwinters in water or infected S. *dulcamara* in the Netherlands**.** Sampling and isolation of *Ralstonia pseudosolanacearum* was performed according to protocols described in EU Regulation 2022/1193. Isolates were identified to species level based on matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) and Sanger sequence analysis of the *egl* locus. *R. pseudosolanacearum* (phylotype I) has a very broad host plant range, including potato, many ornamental and other economically important crops. This highlights the risk for various host plants grown in the vicinity of the geographic locations where *R. pseudosolanacearum* has been found and shows the importance of unraveling the epidemiological parameters of the survival, establishment and spread of *R. pseudosolanacearum* in temperate climates.

A6

Prospecting interspecific pathogenic and growth characteristics on some isolates of *Ralstonia solanacearum*

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The bacterium *Ralstonia solanacearum*, is pathogenic to several plant species, including more than 54 botanic families. In tomato, the resistance to bacterial wilt is strictly quantitative, with few resistant cultivar options, as many of them are derived from lineage Hawaii 7996. From these, thermal amplitude of growth, bacteriocins production and the capacity to cause disease was studied, with emphasis on the isolate CNPH-RS 488, which overcame the bacterial wilt resistance of Hawaii 7996. This isolate was compared to eight other isolates from the same species and biovar, all coded as CNPH-RS, 429, 498, 506, 534, 564, 618 and two additional isolates representing other species/biovars, K60 (biovar 1) and GMI1000 (biovar 3 – *Ralstonia pseusolanacearum*). The growth of all isolates was evaluated in four temperatures: 18°C, 25°C, 33°C and 40°C. Bacteriocin production of all isolates was assessed *in vitro* by visualization of growth inhibition in culture media after pipetting of filtrate solution of each isolate. Evaluation of virulence was assessed by inoculating each isolate on plants of the resistant lineage Hawaii 7996 and on the susceptible lineage L390. CNPH-RS 488 presented typical colonies between 25°C and 33°C. Other isolates presented growth between 18°C to 40°C, although, with this last temperature, colonies were phenotypically distinct. For the bacteriocin production and sensitivity, isolate 488 was inhibited by three other isolates, 498, 534 and 618, while only inhibiting isolate 618. Isolate 498 stood out, presenting a highly competitive capacity, inhibiting four isolates, including 488, while not being inhibited by other isolate's bacteriocins. For virulence response, both isolates, 488 and 564 induced wilt on the resistant lineage Hawaii 7996, whereas all isolates wilted the susceptible L390 plants. The isolate 488, was inhibited by the bacteriocins of several isolates and could not grow on all tested temperatures, suggesting its eventual low adaptability and environmental spread and survival.

A7

Evaluation of the phenotypic and genotypic diversity of *Ralstonia solanacearum* in metropolitan France and the risks for emergence of other species of the *Ralstonia* spp. Complex.

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The *Ralstonia solanacearum* species complex (RSSC) is responsible for bacterial wilt in over 250 plant species. Since 2014, it includes three distinct species: *R. solanacearum* (Phylotype II), *R. pseudosolanacearum* (Phylotypes I and III) and *R. syzygii* (Phylotype IV). Due to its wide geographical distribution, extensive host range and high socio-economic impact, the RSSC is considered as one of the most damaging pests worldwide. As such, the European Union considers these three species as quarantine organisms.
This PhD project, starting from the end of 2022, will enable us to obtain an overview of the genotypic, phenotypic and pathogenic diversity of nearly four hundred RSSC strains isolated in metropolitan France over the past 25 years.
These results, combined with the epidemiological data already collected (date and location of the outbreaks, type of the original samples), will provide information on the origin of the outbreaks (introduction, dissemination and invasion routes) thanks to population genetics approaches and analysis methods such as MLVA, comparative genomics and phylogeography. On the other hand, the pathogenic diversity of the strains (virulence, aggressiveness, host range) will be characterized by confined greenhouse studies on a range of crops and weeds.
These results will also allow us to assess the level of risks associated with other RSSC species in France and more broadly in Europe, and provide the basis for the development of detection tools and epidemiological monitoring of host crops, including seed potatoes.
This PhD project is part of the management context of this quarantine organism, in compliance with European regulations. The expected results will contribute to protect the actors likely to be impacted by these bacterial species, in particular the seed potato industry, but also those producing tomatoes or ornamental plants.

A8

Genomic sequencing of different sequevars of *Ralstonia pseudosolanacearum* strains isolated sunflower.

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The soil-born pathogen *Ralstonia pseudosolanacearum* caused severly bacterial wilt on solanaceous crops in south China. A bacterial wilt on sunflower is a new disease in Guangdong province, south China. The pathogen of sunflower bacterial wilt was identified to be *R. pseudosolanacearum* which includes sequevar 13, 14, 17 and 54. And the strains of different sequevars showed different pathogenicity on the same cultivar of tomato. This study aimed to sequence, assemble, annotate, and compare the genomes of four representative strains RS639, RS642, RS647 and RS650. The four *R. pesudosolanacearum* genomes consist of a circle chromosome and a circle megaplasmid respectively, no small plasmid was found in four stains genomes. The sequence of the four genomes were assembled into different sizes, of which RS639 genome was the largest (5,941,034 bp with 66.85% GC content), RS642 genome was the smallest genome (5,838,575 bp with 67% GC content). A total of 5903 homolog families were identified across genomes of four strains and strain GMI1000. The final core genome comprised 4098 gene families. The similarity of four strains are closely related with each other with the pairwise nucleotide identify (ANI) values ranging from 99.05% ~ 99.71%. We did the comparison analyses of secretion systems. The four strains possess different gene clusters of type Ⅱ secretion systems. There was minimum difference in type Ⅲ secretion systems. Type Ⅳ secretion systems were lost in the genomes of strains RS639 and RS647. The numbers of genes constituting type Ⅳ secretion systems were different between the four genomes. Further, we identified the effectors according to the Ralsto T3E database. The numbers of T3E effectors in four genomes were quite different. A total of T3E effectors found in the four strains is 77, and 51 (67.11%) of which belongs to the core-genome. In a brief, the genome components of different strains isolated from the same host is significantly different, and the data will be helpful for the further study of the pathogenic mechanism of *R. pseudosolanacearum*.

A9

The bacterial wilt of tomato caused by *Ralstonia solanacearum*: an emerging disease in Chile.

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In Chile, *Ralstonia solanacearum* was first reported in potato plants in 1983 and currently is a quarantine disease in the most important potato producing regions located at the south of the country. Tomato, another important host for *R. solanacearum*, is the fourth vegetable with the largest cultivation area in Chile, registering a total of 5,128.1 ha of fresh tomato crops until 2021. In recent years, wilting symptoms has been detected in tomato fields located in different regions in the North and Central Chile. Tomato plants showing yellowing and generalized leaf wilting, stunting, and brown coloration of vascular tissues were collected from tomato fields in the northern Region of Arica y Parinacota (desertic climate) and in central Chile (warm and template climate) in Valparaíso, O´Higgins and Maule Regions. Isolations of typical *R. solanacearum* strains were carried out on both CPG (white mucoid colonies) and TZC solid medium (white mucoid colonies with pink centers). The molecular identification of the strains was carried out by 16S rRNA gene sequencing and PCR analysis with specific primers. The Phylotypes were determined though partial endoglucanase (*egl)* gene amplification, sequencing, and phylogenetic analysis. All isolated strains were identified as *Ralstonia solanacearum* Phylotype IIB sequevar 1 (IIB-1). Pathogenicity assays were performed in susceptible tomato plants following EPPO protocol. The inoculated plants showed severe wilting and bacterial ooze emerging from the inoculation spot after 6 to 12 days. Additional analyses are being carried out to continue the characterization of the strains and to establish the possible origin of this outbreak. These preliminary results will be useful for developing and performing an integrated management that allows controlling losses due to bacterial wilt disease caused by *R. solanacearum* in agricultural tomato and potato crops.

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A10

Genetic diversity and pathogenicity variation of *Ralstonia solanacearum* strains from peanut in Central and Southern China

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Peanut bacterial wilt occurred widely in China, especially in the central and southern regions. To clarify the genetic diversity and pathogenic variation of different *Ralstonia solanacearum* strains affecting peanut in China, the genomic DNA of 95 strains collected from nine peanut growing zones in central and southern China were amplified and sequenced. The results showed that all of the peanut strains belonged to phylotype I (Asia type). The phylogenetic tree based on the nucleotide sequences of *egl* gene and reference strains of *R. solanacearum* was generated and the involved strains were grouped into different sequevars. Strains isolated from eight zones in five provinces belonged to sequevar 14, while the strains from Guangxi Province belonged to sequevar 48. It suggested that the level of genetic diversity of *R. solanacearum* strains affecting peanut was generally low in central and southern China. The representative strains of each zone were tested for their pathogenicity on susceptible peanut genotypes, and pathogenicity variation was found among these strains. According to disease index, the strains were classified into highly, moderately and weakly virulent, and about 67% of the strains were identified as highly virulent strains.

A11

Result of whole genome sequence-based characterization of eight *Ralstonia solanacearum* isolated in Georgia.

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Ralstonia solanacearum is a soil-born Gram-negative bacterium, the causative agent of globally dispersed bacterial wilt disease, infecting an unusually wide range of plant species. The harm is particularly serious in economically important crops, such as tomatoes, potatoes, and tobacco. We study the whole genome sequence of the test isolates on the Illumina Miseq platform (Illumina, Inc.) per the manufacturer's instructions. Here we report the whole genome sequence-based analysis of eight strains of *R. solanacearum* (GEO_6, GEO_55, GEO_57, GEO_81, GEO_96, 31 GEO_99, GEO_230 and GEO_304) isolated in Georgia causing bacterial wilt in the country.

DNA of Georgian *R. solanacearum* isolates was sequenced. 16S rRNA genes and endoglucanase precursors were used to establish relatedness between Georgian isolates and reference strains of *R. solanacearum.* Additionally, complete genome pairwise comparison such as Average Nucleotide Identity (ANI) was used in our study. 16S rRNA and endoglucanase gene sequences of Georgian isolates are similar to that of strain *R. solanacearum* UY031, which is found in Uruguay. Georgian isolates have about 99.99–100% ANI with one another and strain UY031. According to 16S rRNA, endoglucanase gene and ANI Georgian isolates and strain UY031 are closely related to other American strains (IBSBF1503, Po82, and UW163) and forming phylotype IIB.

The comparison of the genome of Georgian isolates to strain from phylotype II shows i) besides the high similarity of Georgian isolates to UY031 strain deficient of UY031 genes is found on the chromosome and mega plasmid of Georgian isolates. ii) Sequence similarity of some consecutive genes (that do not exist in the UY031 strain) of Georgian isolates to strains CFBP2957, Po82, IBSBF1503, and UW163. Thus, Georgian isolates should be considered as new strains of R. solanacearum.

The results suggest that all Georgian isolates belong to phylotype IIB and should be originated in South America. The genome sequence data would be a valuable resource for the evolutionary, epidemiological studies and quarantine of this phytopathogen.

A12

Genetic diversity of the type III effector RipAX2 in the *Ralstonia solanacearum* species complex and its impact on the deployment of eggplants carrying the *EBWR9* resistance locus in the South-West Indian Ocean

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The *Ralstonia solanacearum* species complex is responsible for the bacterial wilt disease on many food crops with high economic and food potential. This disease represents one of the major constraints to the sustainable production of Solanaceae crops, with heavy socio-economic consequences for small farmers in the South-West Indian Ocean islands (Comoros, Madagascar, Mauritius, Mayotte, Reunion, Rodrigues and Seychelles). The measures commonly used to limit losses are the implementation of regulations on the circulation of plant material, crop rotation and use of healthy seeds. These prophylactic methods are not totally effective and the use of resistant varieties remains the most promising strategies to control this disease.

We evaluated the effectiveness of bacterial wilt resistance of the eggplant AG91-25 carrying the *EBWR9* locus (Salgon et al., 2017). This resistance is conferred by the recognition of the type III effector RipAX2 (Morel et al., 2018). A study of RipAX2 diversity was performed by *in silico* analysis based on 550 genomes assemblies from public databases and by targeted gene sequencing of a collection of 807 global strains. We identified different alleles of RipAX2 and selected a subset of strains representative of this diversity that were inoculated on AG91-25 and MM738 used as a susceptible control. Strains displaying minor polymorphisms of RipAX2 (e.g. amino acid substitutions) were controlled by AG91-25 while those with major variations (e.g. insertion sequence event, frameshift, premature stop codon) were virulent and triggered bacterial wilt symptoms. The phenotyping of these strains complemented with the reference sequence of *RipAX2* confirmed the involvement of this effector in the interaction with AG91-25.

Finally, we performed a geographical mapping of the virulence profiles of all the strains analyzed in this study, to define a rational deployment plan of the *EBWR9* resistance limiting the risks of resistance breakdown in the agroecosystems of the different South-West Indian Ocean islands.

A13

Ralstonia solanacearum preferential colonization in the shoot apical meristem explains its pathogenicity pattern in tomato seedlings.

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Ralstonia solanacearum causes a lethal bacterial wilt disease in many plants by colonizing the vascular tissues of the hosts. Upon inoculation in tomato seedlings either through leaf or root, the wilting symptoms initiate at the apical region and then proceed downward along the shoot. The systematic order of the disease initiation and progression in the host independent of the site of pathogen inoculation though interesting, is yet to be investigated. To understand it, here we have done a systematic study of the pathogen localization by gus staining the inoculated tomato seedlings from zero days post inoculation (DPI) to 5 DPI at every 24 h duration. Interestingly, in both inoculation modes, on the first DPI itself the pathogen colonization was observed at the apical meristem as well as the cotyledon leaves, where the disease initiates. As the disease progressed, the colonization of the pathogen towards the lower region of the shoot was observed. Disease consistency and pathogenicity magnitude were observed to be higher in leaf inoculation than that in root inoculation. Several *R. solanacearum* transposon induced mutants that were reduced in virulence by root inoculation but proficient by leaf inoculation were obtained. By GUS staining it was observed that these mutants were deficient in localizing in the shoot region by root inoculation. Our study indicates that the apical meristem and the cotyledon leaves are the regions to be colonized first in the inoculated tomato seedlings, which attributes for the initiation of the wilt disease from this region.

Travel Award

A14

Identification of the *RSc1155* gene involved in cinnamic acid chemotaxis in plant infection by *Ralstonia solanacearum.*

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Chemotaxis towards chemical signals contained in the exudates is very important for pathogenic bacteria recognition, colonization, and infection of host plant. In this study, we observed chemotaxis, motility and biofilm formation toward cinnamic acid by *Ralstonia solanacearum* CQPS-1. At 100 and 150 μM concentrations, cinnamic acid was found to be a strong chemoattractant for inducing the chemotactic response and swarming motility of *R. solanacearum*. Transcriptome analysis of cinnamic acid-exposed *R. solanacearum* showed that *RSc1155*, a methyl-accepting chemotaxis protein (*mcp*) gene, was involved in signal transduction in *R. solanacearum*. Then, we constructed a single *mcp RSc1155* gene deletion mutant of *R. solanacearum* and found that Δ*RSc1155* mutant was defective in chemotaxis, motility and biofilm formation to cinnamic acid, respectively. Further pot experiment showed that △*RSc1155* mutant strain weakened responses to cinnamic acid and displayed significantly less infectivity to tobacco plants than the wild type, indicating that cinnamic acid may be an important chemical signal in the interaction between plant and CQPS-1. These findings suggested that RSc1155-mediated chemotaxis to cinnamic acid, facilitates *R. solanacearum* motility and infection to tobacco plants.

A15

Participation of *Ralstonia solanacearum* catalases in the plant-pathogen interaction.

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Ralstonia solanacearum (*Rso*) is a saprophytic soil bacterium that causes the disease known as bacterial wilt in more than 200 plant species worldwide. The accumulation of reactive oxygen species (ROS) constitutes one of the first plant defense responses against infection by pathogens. To overcome this barrier, microorganisms have various detoxification enzymes capable of eliminating these ROS, and among these enzymes, catalases are central components of detoxification pathways. *Rso* GMI1000 genome revealed the presence of genes encoding putative catalase enzymes such as the monofunctional catalase KatE, and the catalase-peroxidase enzymes KatG encoded by *RSc0775/RSc0776*. The present work aims to study and characterize the physiological role of the KatG enzyme. The *Rso* Δ*katG* and the Δ*katEkatG* double mutant strains were generated and were physiologically characterized. The analysis of *Rso* soluble protein extracts in native polyacrylamide gels revealed for catalase activity allowed the band identification corresponding to the KatG enzyme. In addition, the detection of peroxidase activity in non-denaturing gels allowed us to corroborate its catalase-peroxidase bifunctional enzymatic nature. The Δ*katG* mutant strain did not show differences in catalase activity levels compared to the wild type strain, however, exhibiting higher inhibition halos in assays against H_2O_2. On the other hand, the double mutant strain did not present detectable levels of catalase activity and exhibited considerably higher inhibition halos on BG-Agar Soft plates. Furthermore, the protein expression levels of *Rso* catalases were analyzed when exposed to methyl viologen, and it was observed that the KatG enzyme increases its activity when faced with this oxidizing agent. These results suggest that *Rso* catalases do not play an essential role in the early oxidative response triggered by plants against infection.

A16

Calcium increases bacterial wilt resistance in potato and decreases *Ralstonia solanacearum* virulence.

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Potato (*Solanum tuberosum*) is one of the most important widespread hosts of *Ralstonia solanacearum*, the causal agent of bacterial wilt. Uruguay has been conducting a national potato breeding program focused on the development of germplasm with resistance to *R. solanacearum.* The aim of this work is to assess mineral compounds found in the xylem sap and plant tissues involved in the interaction between *R. solanacearum* and germplasm generated in the breeding program. The mineral content of xylem saps, roots, stems and leaves of selected clones was evaluated by inductively coupled plasma with optical emission spectroscopy. A correlation between mineral concentration and genotype resistance in xylem sap and stem was found, with higher levels of calcium, magnesium, phosphorus, potassium, and sulfur in resistant genotypes. Calcium was selected to continue virulence traits evaluation. Growth curves were performed in rich medium and minimal medium supplemented with calcium. In minimal medium calcium decreased *R. solanacearum* growth. Calcium also decreased biofilm formation and twitching motility. The use of microfluidic chambers was optimized as a new model to study the bacteria under flow conditions. Differences were observed in multiplication, attachment, biofilm formation and cellular morphology in rich medium in comparison to minimal medium. Calcium was also supplemented in media in microfluidic chambers and a reduction in bacterial attachment and biofilm formation was observed. To evaluate the effect of calcium on bacterial wilt resistance, plants of a susceptible and a resistant genotype were treated with calcium by watering, and then inoculated with bacteria. In the susceptible genotype a delay in disease progress was observed with a significant decrease in symptoms when plants were treated with calcium and in the resistant genotype plants remained asymptomatic. This study contributes to the understanding of mechanisms of adaptation and virulence of this important pathogen and evaluates possible tools for integrated management for bacterial wilt control.

A17

Evaluation of sources of resistance against *Ralstonia solanacearum* and *Clavibacter michiganensis* in tomato

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The cultivated tomato, *Solanum lycopersicum* (formerly *Lycopersicum esculentum* Mill.) is one of the most consumed vegetables worldwide, ranking second in terms of production and cultivated area. *Ralstonia solanacearum* (Rs) and *Clavibacter michiganensis* (Cm) are among the most devastating pathogens in solanaceous crops. In Uruguay, Cm is identified by growers as the major problem if the disease infects the crop. At the same time, Rs currently does not represent a risk, but in Brazil, Rs is considered one of the main problems for tomatoes in the field and greenhouse. In this context, the objective of this research was to evaluate the resistance levels of a pool of 37 accessions of the section *Lycopersicum* to both pathogenic bacteria, with experimental inoculation of virulent strains. The inoculation with Cm was carried out in multicell trays, using 45-day-old tomato plants. The stem was punctured above the petiole of the first true leaf with 1 µl of suspension adjusted to 10^8 cfu/mL. For the inoculation with Rs, 40-day-old tomato plants grown in multicell trays were used. At the time of transplantati, the 4 sides of the clod were sprayed with a suspension of 10^7 cfu/ml. In both trials, four evaluations were carried out, where a visual scale was used and severity levels were assigned: susceptible, moderately susceptible, moderately resistant, and resistant. Of the total number of accessions evaluated, seven were resistant or moderately resistant to both bacteria, belonging to *Solanum lycopersicum, and Solanum peruvianum.* In particular, OHIO 4013 is reported as a source of resistance to other bacterial diseases. This finding validates the preliminary data of our evaluation, where a high level of resistance for Cm and Rs was identified. Crosses between OHIO 4013 and a susceptible accession were made to study the inheritance of resistance.

A18

Improving nitrogen fixation capacity of bacterial wilt-resistant peanut genotypes by discovering and integrating dominate genes for nodulation

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Development and employment of bacterial wilt (BW)-resistant varieties are highly crucial for controlling this important soil-borne disease caused by *Ralstonia solanacearum* in peanut (*Arachis hypogaea* L.). However, the yield potentials of the available BW-resistant peanut genotypes including landraces and improved cultivars are significantly lower than that of high-yielding susceptible ones, which largely limits the peanut productivity in the BW-infested regions. Relatively low symbiotic nodulation capacity in the BW-resistant genotypes is an important reason for their low productivity. The nodulation in peanut is controlled by two dominant genes, but most BW-resistant genotypes only possess one gene. In this study, two special peanut lines (Zhonghua 10 and ICG 12625) each harboring one dominant gene for nodulation were identified and their recombinant inbred lines (RILs) (Zhonghua 10×ICG 12625) were used in mapping QTLs for nodulation. A high-density genetic map was constructed, and two major QTLs (*qPNA08* and *qPNB07*) were identified. Meanwhile, the RILs were also used to map QTLs for BW resistance from ICG 12625. Further efforts will be made for precisely introgress the complementary dominant nodulation genes into BW-resistant genotypes to enhance productivity.

A19

Soil properties drives bacterial community assembly in tobacco rhizosphere affecting bacterial wilt disease.

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It is vital to make a good understanding of microbial community assembly in plant rhizosphere for the control of bacterial wilt disease and the stability of farmland ecosystems. Bacterial communities of tobacco rhizosphere and soil properties from the major eight different ecotopes of tobacco-planting in China with different occurrence degree of tobacco bacterial wilt disease were investigated via high-throughput sequencing and bioinformatics. The most abundant bacterial class were *Actinobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria* and *Thermoleophilia*. The composition of the bacterial community was clustered according to ecotopes, and the similarity of the bacterial community among samples were significantly negative related to spatial distance. Co-occurrence networks of bacterial interactions indicated a higher proportion of positive links between bacterial genus inferred relationships of fundamental cooperation among the bacterial community. The ecotope of tobacco-planting with higher occurrence degree of tobacco bacterial wilt possessed fewer beneficial bacteria like Firmicutes, *Bacillus* and *Pseudomonas* and more *Ralstonia* in tobacco rhizosphere like WQM (Wuling-Qinba mountains), while, higher modularity of co-occurrence network and stronger interaction between bacteria in tobacco rhizosphere were detected in the ecotope of YMH (Yimen hill) and NEP (Northeast plain), and *Micromonospora*, *Bryobacter* and *Arenimonas* were identified as network hubs or module hubs, which played important roles in stabilizing bacterial co-occurrence in the tobacco rhizosphere, and it was the characteristics but relative abundance determined the roles. Results of redundancy analysis (RDA) showed that pH, AvailFe (available iron), ExchMg (exchangeable magnesium) and AvailMn (available manganese) remarkably dominated to the bacterial community assembly in tobacco rhizosphere. Together, we found that the homogenization and habitat specificity of bacterial community assembly in tobacco rhizosphere were drove by soil pH, AvailFe, ExchMg and AvailMn. Moreover, Firmicutes, *Bacillus*, *Pseudomonas*, *Micromonospora*, *Bryobacter* and *Arenimonas* may play important roles in the bacterial community of tobacco rhizosphere as well as suppression of tobacco bacterial wilt disease.

A20

Discovery of a novel QTL on chromosome B03 for bacterial wilt resistance in peanut variety ICG12625

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Peanut (*Arachis hypogaea*) is an important oilseed crop worldwide. Utilization of genetic resistance is the most economic and effective approach to control bacterial wilt, one of the most devastating plant diseases, in peanut production. To accelerate the genetic improvement of bacterial wilt resistance (BWR) in peanut breeding programs, quantitative trait locus (QTL) with BWR of a few resistant varieties has been identified and clustered on chromosome B02. In this context, we deployed linkage mapping to identify genomic regions and candidate genes for BWR in a resistant genotype ICG12625. The recombination inbred line (RIL) population (140 progenies) from the cross Zhonghua 10 × ICG12625 was used in BWR evaluation across four environments. Based on whole-genome resequencing, 2701 recombination bins were identified in the RIL population and a high-density genetic map was constructed, which covered 1469.56 cM with an average inter-marker distance of 0.54 cM. QTL mapping identified a stable QTL (*qBWRB03*) on chromosome B03 with 10.58-12.01% phenotypic variation explained (PVE) across the four environments. Therefore, the major and stable QTL *qBWRB03* on chromosome B03 could be deployed together with the previously reported QTLs on B02 in genomics-assisted breeding (GAB) to develop improved peanut varieties with enhanced BWR.

A21

Screening a core collection of potato wild relatives from Uruguay for bacterial wilt resistance.

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Potato wild relatives, *Solanum commersonii, S. malmeanum* and *S. chacoense,* are naturally distributed in Uruguay, Argentina and Brazil. The aim of this study was to find new sources of resistance to bacterial wilt by screening a core collection of potato wild relatives native to Uruguay. These genotypes were multiplied to install an experimental design with randomized blocks; 2 independent experiments were carried out in a quarantine solarium. Inoculum was prepared with an aggressive *R. solanacearum* strain (UY031) belonging to the phylotype IIB, sequevar 1. Inoculations were performed using a bacterial suspension per plantlet of 10^6 cfu·g^{-1} of soil and producing root damage. Disease progression was registered regularly from the onset of the disease until 38 days after inoculation using a scale ranging from 0 (asymptomatic plant) to 4 (all leaves wilted). Resistance level was calculated by the area under disease progress curve (AUDPC) based on the average wilt scoring for each clone. Latent infections were detected by sampling basal stem sections from asymptomatic plants, through direct bacterial culture and BIO-PCR. The statistical analysis concluded that the 3 species behaved similarly, and the evolution of symptoms was similar between different genotypes. Moreover, there were differences between the 2 experiments: in the first, significant differences were found between the genotypes, while in the second all genotypes were affected without distinguishing different levels of resistance. Within the genotypes of the core collection, A11P1, C14P1, C6P1, RN3P1 stood out, since the appearance of their symptoms was delayed, which led to a less development of the disease. Additionally, the latency evaluation assay showed that certain genotypes such as A11P1 did not present this type of infection. Analyzing all the results, we conclude that there is great variability in resistance levels in this core collection. Accessions with high levels of resistance and low latency can be identified for further use in introgressive breeding.

A22

Evaluation of resistance to bacterial wilt in advanced potato (*Solanum tuberosum* L.) germplasm.

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Bacterial wilt is the second most important disease affecting the potato crop. Commercial varieties do not have considerable levels of resistance to this pathogen, but there are potential sources of resistance in the potato germplasm, including wild relatives of potato native to Uruguay. One of the aims of the National Potato Breeding Program in Uruguay has been to incorporate germplasm resistant to bacterial wilt and to combine resistance sources by hybridization and backcrossing. The aim of this work was to characterize resistance to bacterial wilt in advanced germplasm from the National Potato Breeding Program and other promising materials from different origins. Two methods of evaluation were used: inoculation in a phytotron under controlled conditions and in a greenhouse resembling field conditions. A Randomized Complete Block design was used for both trials. Symptoms were monitored periodically by visual assessment on the plant and asymptomatic tubers were evaluated by BIO-multiplex PCR to detect latent infections. The genotypes evaluated showed different levels of resistance, and all showed latency. An analysis of variance (ANOVA) showed significant differences among genotypes, which were grouped into two categories according to Tukey's test. A positive correlation of the two methods was observed using Pearson's coefficient. Spearman's coefficient showed that there is no linear association. Given the difficulties in controlling the pathogen by other means, the identified resistant materials have great potential within an integrated control strategy and potential for crosses that also combine resistance to other important diseases such as late blight or common scab.

A23

Optimizing screenings to simplify phenotyping and dissect the genetic architecture of bacterial wilt resistance in potato wild relatives from Uruguay using GWAS.

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Potato wild relatives distributed in Uruguay are reported to show resistance against bacterial wilt, but little is known about the underlying genetic architecture of the different responses they display. Because large-scale phenotyping to find genome associations can be challenging, the aim of this study is to optimize the screening of a large collection of potato wild relatives representative of the different uruguayan agro-climatic regions, for resistance to bacterial wilt. We aim to more efficiently obtain robust and repeatable phenotypic data for genome-wide association studies (GWAS). A total of 190 genotypes, belonging to three species and with a differential response in terms of resistance, were multiplied in vitro. Seedlings were further multiplied from apical cuttings using an autotrophic hydroponic method to obtain sufficient plant material for the bacterial wilt resistance assay. We evaluated all the accessions simultaneously in a phytotron under controlled conditions, in a Randomized Complete Blocks Design with three blocks. Temporal blocks were used, i.e. Block 1 was inoculated and evaluated during the first month, Block 2 during the second month and Block 3 during the third month. Inoculum was prepared with an aggressive *R. solanacearum* strain (UY031) belonging to the phylotype IIB, sequevar 1 and inoculated at a concentration of 10^6 cfu·g^{-1} of soil, previously producing root damage. Disease progression was registered regularly from the onset of the disease until 31 days after inoculation using a scale ranging from 0 (asymptomatic plant) to 4 (whole plant wilted). Resistance level was calculated by the area under disease progress curve (AUDPC). Preliminary results indicate that we could detect great variability in the response against bacterial wilt in this collection, which means it has great potential for successful association mapping and that new sources of resistance can be identified for further use in introgressive breeding.

B1

Reservoir hosts of *Ralstonia solanacearum*: a key element of the fight against bacterial wilt.

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Solanum dulcamara and *Solanum nigrum* are two winter reservoirs of *Ralstonia solanacearum* around the world. In the UK, the bacterium overwinters in these weeds without causing any damage or symptoms. During the summer, it is released from the plant roots to the river from where it can contaminate other fields via irrigation water. To understand the mechanisms that allow *S. dulcamara* and *S. nigrum* to be invaded by *R. solanacearum* without showing any symptoms, we have obtained two highly contiguous plant genomes by using Nanopore technologies and compared them against the genomes of susceptible tomato, eggplant and potato hosts. We have identified the orthologous genes between these species, and those only present in *S. dulcamara* and *S. nigrum* which can confer an advantage during *Ralstonia* infections. We will also assess the gene reprogramming differences using mRNA-Seq data and RT-qPCRs, between tomato and *S. dulcamara* after *Ralstonia* Phylotype IIB infection. These analyses will allow us to identify genes associated with resistance and tolerance mechanisms that can be later used to produce resistant tomato cultivars which can survive this deadly pathogen.

B2

Single-cell RNA-seq strategy to identify effector-targeted plant cells.

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Ralstonia solanacearum is one of the most important plant pathogenic bacteria according to its aggressiveness, large host range (more than 394 plant species), broad geographical distribution and long persistence in soil and water environments. *Ralstonia's* distribution in plants is variable and tissue specific during infection. *Ralstonia* enters roots through wounds or natural openings, multiplies in the intercellular spaces and causes plasmolysis of epidermal cells. The pathogen invades plant via xylem vessels resulting in systemic spread. *Ralstonia* can also break free from the xylem, colonizing the apoplast. *Ralstonia* translocates proteins, called effectors, through the type III secretion system into plant cells. Effectors can manipulate plant metabolism and defense and facilitate disease development. It remains unknown which cell types are targeted by *Ralstonia* for effector delivery and individual plant transcriptional responses in different tissues and cell types. We hypothesize that variation in cellular response between effector-targeted and untargeted cells shape plant responses and disease outcomes. In this project, we will use an artificial transcription activator-like-effector (arTALE) expressed from *Ralstonia* to activate a plant reporter gene in effector-targeted cells. The targeted cells can be identified by expression of the reporter gene. With this system, we will perform a single-cell RNA sequencing (scRNA-seq) of transcriptional responses in effector-targeted and untargeted cells in different tissue types during *R. solanacearum* infection. The successful completion of these experiments will allow us to determine cell type specificity in effector delivery and tissue specific responses to *Ralstonia* infection.

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Travel Award

B3

Virulence assessment of *Ralstonia solanacearum* (phylotype II) isolated from ornamental *Rosa* spp. plants.

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Ralstonia solanacearum (phylotype II) isolates PD 7421 and PD 7394, found in 2018 in asymptomatic ornamental rose (*Rosa* spp.), were assessed for their virulence in two rose cultivars ("Armando" and "Red Naomi"). After stem inoculation, plants were incubated at 20°C and 26°C for 106 days. Disease severity was assessed during this period and re-isolations were performed from symptomatic plants, in order to confirm the *R. solanacearum* infections. Plants showing no symptoms at 106 dpi were also included for the re-isolations in order to evaluate the presence of *R. solanacearum* in a latent state. The identity of re-isolates exhibiting typical colony morphology was confirmed by MALDI-TOF MS analysis. While rose inoculated with *Ralstonia pseudosolanacearum* (phylotype I) isolate PD 7123, a reference strain previously shown to result in high disease severity in ornamental rose, showed severe symptoms at both temperatures, no typical symptoms were acquired for *R. solanacearum* isolates PD 7421 and PD 7394 on the rose cultivars included in this study, irrespective of the temperature.
R. solanacearum (phylotype II) is known as a major potato pathogen, causing brown rot in potato. Whole genome multilocus sequence typing analysis demonstrated that the phylotype II isolates from rose were closely related to phylotype II isolates previously found in seed potato and surface water in the Netherlands. Because of this close genetic relatedness, the virulence of PD 7421 and PD 7394 was also assessed in potato plants, where both isolates caused severe disease symptoms on both the above ground plant and the daughter tubers.

B4

Relative expression of *βhpmeh* gene in transgenic events of the potato variety 'Desiree' related to resistance to bacterial wilt caused by *Ralstonia solanacearum.*

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Bacteria wilt is caused by *Ralstonia solanacearum* (RS) species complex (E.F.Smith) in a wide range of crops, the most susceptible crops being: potato, tomato, eggplant, pepper, banana and groundnut. In potato, bacterial wilt has spread to most potato producing countries. RS regulates expression of virulence factors such as extracellular polysaccharide (EPS) and endoglucanases by quorum sensing. The production of these virulence factors is activated by the accumulation of the quorum signal 3-hydroxi-palmitate methyl ester (3-OH PAME), which can be hydrolyzed by the enzyme β-hydroxi-palmitate methyl ester hydrolase (βHPMEH), found in soil inhabiting bacteria. By expressing βHPMEH in plants it may be possible to prevent RS to produce virulence factors, thus preventing disease development. We previously transformed potato plants with *βHPMEH* codon optimized for expression in plants and fused with an extracellular excretion peptide, under control of the Cauliflower mosaic virus 35S promotor or xylem specific GRP1.8 promotor, that could inhibit activation of virulence genes that are involved in the disease process by sequesting 3-OH PAME accumulation. In this study we evaluate the expression of the *βhpmeh* gene in transgenic events of the potato variety 'Desiree' (CIP800048) related to the level of resistance against the strain CIP-204 phylotype II Bv2A of *R. solanacearum* under greenhouse conditions. For each transgenic event we analyzed the phenotypic response (wilt incidence, latent infection and the area under the disease progress curve [AUDPC]) and reverse transcription quantitative PCR (RT-qPCR) was performed to determine the relative expression level of *βhpmeh* in the same events. Six transgenic events (GRP3.11Dc, GRP3.45Nc, GRP7.15dc, II10.29, II4.16Adc and II 6.3Dc) were analyzed during different seasons and from different planting materials (tubers or *in-vitro* plantlets). Cruza-148 was used as a resistant control and showed statistically significant reduced wilting, AUDPC and infection comparted to the susceptible non-transgenic control 'Desiree', as expected. All transgenic events also showed a reduction in infection and disease parameters, but this was largest and significant only in three events. Resistance however did not seem to be strictly correlated to gene expression as determined by RT-qPCR, as the most resistant events were not necessarily the ones with the highest level of *βhpmeh* expression.

B5

Bacterial wilt disease of *Eucalyptus pellita* in Indonesia: disease trigger factors, pathogen and host plants diversity.

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The productivity of forest plantations such as *Eucalyptus pellita* in Indonesia is low, one of which is severely affected by bacterial wilt. However, studies of bacterial wilt on *E. pellita* in Indonesia are still inadequate, particularly regarding to the integration of site and genetic factors; thus, a comprehensive study is required. The main objective of this study was to analyze the dominant variables that can trigger the disease; identify and describe the diversity of the causal agent; evaluate the resistance of eucalyptus and analysis SSR markers related to bacterial wilt resistance. Bacterial wilt epidemic is influenced by silvicultural techniques, soil properties and climate. Early growth of eucalyptus plants is a critical period for disease epidemics, especially in susceptible clones and root malformations occur. Soil texture and rainfall have a strong correlation with the incidence of bacterial wilt. The causal agent is *Ralstonia pseudosolanacearum* and has high variability. Genotypic analysis based on the *egl* gene showed that all strains belonged to phylotype 1 and were divided into four existing sequevars in the world and one new sequevar that had never been reported. Clone resistance test results in the growth chamber correlated strongly with clone resistance in the field on a broad scale. There is a QTL map related to resistance to bacterial wilt. The position of the QTL is at locus 240 which is located at 22.6 cM from the EMBRA21 marker. The use of resistant clones, in combination with disease-free seedlings, maintaining normal roots through proper silviculture practice, suppressing the pathogen inoculum to the possible lowest level in the soil, and balanced plant nutrition is an appropriate way of controlling bacterial wilt in eucalyptus plantations. This comprehensive research on the *Ralstonia-Eucalyptus* pathosystem can be used as basic information to develop adaptive disease management strategies.

B6

Microbial community physiological profiles and isolation of *Ralstonia solancearum* biocontrol agents of field-growing tomato plants

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Both rhizosphere and endophytic plant growth promoting bacteria can offer benefits to the host plant, such as protection against *Ralstonia solanacearum* and favor ecosystem ecosustainability. Severity of the wilt disease may be associated with changes in composition of the rhizosphere and endophytic microbial communities associated with the host. *R. solanacearum* could alter plant microbial communities. We characterizedfunctional diversity of both microbial communities associated with tomato plants growing at field conditions andobtained isolatesto select biocontrol agents. Tomato plants were sampled in two fieldslocated in the horticulturalproductivebelt of La Plata, Buenos Aires, Argentina.Several dilutions of the stems and roots+rhizosphere samples of healthy, diseased, and intermediate plantswere cultured on*Pseudomonas* Agar and Nutrient Agar + glucose (NAG). In the latter medium,some sample dilutions werecultured after heating them at 80°C for 30 minutes to isolate potential endospore formingisolates. Besides, the community level physiological profiles (CLPP) and the diversity index H of Shannonwere obtained through analysis of principal components (PCA) and discriminant.In total, 443 isolates were obtained. From those 14.% and 37, 5% were from samples treated without and with heating cultured in NAG medium, respectively. In *Pseudomonas*agar medium,48.5% were obtained. Gram staining showed that 229 and 214 isolates were positives and negatives, respectively. The isolates underwent a contrast test in differential BG medium for *R. solanacearum*, and 75% of them showed similar colonies to this phytopathogen. So, it is necessary to explore other options to perform*in vivo*antagonism experiments. PCA has shown that the percentages of explained variance werelower than in discriminant analysis, Differences were observed between the CLPP of the microbial communities associated with the plants of the two fields. Hindex of microbial communities associated with diseased plants and stems were lower thanthose in healthy plants and roots+rhizosphere samples which could be less susceptible to the presence of the phytopathogen.

Travel Award

B7

Effect of conservation by lyophilization on survival and *in planta* biological control of lytic bacteriophages of *Ralstonia solanacearum*

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Ralstonia solanacearum is a pathogenic bacterium that causes bacterial wilt in solanaceous and ornamental plants. Biological control of this disease is a strategy of great interest, as staple crops for human consumption are frequently concerned. Three bacteriophages (phages) with specific lytic activity against *R. solanacearum*, vRsoP-WF2, vRsoP-WM2 and vRsoP-WR2, were isolated in Spain, and their use for the prevention and/or control of the bacterial wilt disease was patented. However, there was no information on their survival and lytic activity after being subjected to a preservation method for commercialization such as lyophilization (freeze-drying). Therefore, the viability and stability of vRsoP-WF2, vRsoP-WM2 and vRsoP-WR2 lyophiles obtained with various cryoprotectants and their biocontrol capacity against a Spanish strain of *R. solanacearum* in tomato plants were tested. The results regarding the viability and stability of the phages after lyophilization were satisfactory, maintaining high titers throughout the period, and especially when they were lyophilized with 0.5 M trehalose, for at least 90 days. Regarding the biocontrol in the plant, the percentages with the cocktail of the three lyophilized phages after 4 weeks were higher than 50%, only slightly lower than the 65% obtained in plants treated with the non-lyophilized phage cocktail. Control plants inoculated only with phages did not develop any symptom of the disease, indicating their safety and suitability as biocontrol agents. Data provided in this work allow us to increase knowledge about the viability and stability of the lyophilized vRsoP-WF2, vRsoP-WM2 and vRsoP-WR2 phages and their interaction with *R. solanacearum*, as well as to confirm lyophilization as a conservation method for the phages for later processing, commercialization and use in natural conditions.

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B8

Characterization of *Solanum lycopersicum* defense responses to biocontrol with lytic *Ralstonia solanacearum* bacteriophages

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Bacterial wilt caused by *Ralstonia solanacearum* is a devastating disease responsible for serious economic losses in staple crops around the world. Agrochemical control methods have variable efficacy and a high environmental impact, making it necessary the development of innovative, ecological and safe control strategies. We have recently patented the use of three lytic bacteriophages (phages) of *R. solanacearum* for biocontrol of bacterial wilt in environmental water and in plant. However, the response of the plant to the pathogen-phage system remains unknown. In this work, we have analyzed plant defense responses to biocontrol with a cocktail of these three phages using their application in the stem. The biocontrol assays were carried out in tomato plants of the susceptible cultivar Rome by co-inoculating the pathogen and the phage cocktail in the stem. The plant defense responses to biocontrol were analyzed against hormones involved in the main defense pathways and phenolic compounds involved in the defensive system. The results have shown that the phage cocktail is responsible for significant reductions in bacterial wilt in the tomato plants, and that the plant uses various defense responses against *R. solanacearum*.

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B9

Genomic and phylogenetic characterization of *Ralstonia solanacearum* bacteriophages useful for biocontrol in irrigation water and in plant

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Ralstonia solanacearum, the etiological agent of bacterial wilt, is one of the most devastating phytopathogenic bacterial species. Bacteriophage viruses (phages) able to lyse specifically the target bacterium can be promising biological control agents that allow sustainable agrosystems. Three lytic phages of *R. solanacearum*, vRsoP-WF2, vRsoP-WM2 and vRsoP-WR2, formerly isolated from water from different Spanish rivers, have demonstrated their ability to biocontrol the pathogen populations both in environmental water and in plants, and their activity was patented. However, their genomic characterization was necessary to deepen in the knowledge of their biology. In this work, the complete genomes of the three phages were sequenced, and bioinformatic analyses were carried out, as well as phylogenetic comparisons with a selection of phages active against *R. solanacearum* and the closely related plant pathogenic species *R. pseudosolanacearum*. The results revealed that the genomes of these phages range from 40,688 to 41,158 bp with almost 59% GC content, and there were 52 ORFs in vRsoP-WF2 and vRsoP-WM2, and 53 in vRsoP-WR2 but, with only 22 or 23 predicted proteins with functional homologues. Among them, two lysines and one exopolysaccharide depolymerase. This type of depolymerase has been identified for the first time in *R. solanacearum* phages. These three phages belong to the same new species within the genus *Gyeongsanvirus*, of the *Autographiviridae* family (former *Podoviridae*). These new genomic data will contribute to a better understanding of the abilities of these phages to damage *R. solanacearum* cells and, consequently, to an improvement in the biological control of the bacterial wilt disease.

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B10

Green control technology and products innovation of new strategies based on biological barrier against tobacco bacterial wilt disease.

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Ralstonia solanacearum represents one of the most devastating plant bacterial pathogens, infecting more than 250 plant species and causing bacterial wilt worldwide. Tobacco bacterial wilt disease causes serious economic losses in China, and the limitation of control methods may aggravate the harm of bacterial wilt in agriculture. Thus, development of the potential green control strategies and theories for bacterial wilt is highly demanded. In this study, we clarified the microbial communities' characteristics of rhizosphere and bulk soil associated with tobacco bacterial wilt in China and figured out the colonization rules of beneficial bacteria in tobacco rhizosphere. Soil pH, tobacco root exudates, medium and trace elements, and aluminum ions could affect the occurrence of tobacco bacterial wilt and construction of plant root biological barrier. Based on our studies, we proposed the three-layer biological barrier theory of plant defense mechanism against *R. solanacearum*, the first biological barrier was constituted with beneficial microbiome of bulk soil, the second biological barrier depend on beneficial rhizospheric microbiome, and the third biological barrier was constituted with beneficial endophyte. The key to control bacterial wilt by enhancing three biological barriers is improving the relative abundance of beneficial microbiome, and the first and second biological barrier are the most feasible and efficient breakthrough points. Furthermore, we proposed green control strategies based on theory of the four balances included acid-base balance, nutrient balance, micro-ecological balance, and host resistance balance. Based on the theory, we have screened hundred efficiency benefic microbes and control agents for controlling tobacco bacterial wilt. These technologies have used in Chongqing, Sichuan, Yunnan, Hubei, Guizhou provinces for millions of acres, and increased the outcome of tobacco for three hundred million yuan. Therefore, the breakthrough of the three-layer biological barrier and four-balances technologies support the important theory and technology for sustainable development of tobacco and other crops in agriculture.

B11

Isolation and characterization of a jumbo *Ralstonia*-infecting phage with promising biocontrol potential

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Bacterial wilt caused by *Ralstonia solanacearum* is one of the most devastating bacterial plant diseases in the world and is extremely difficult to control. As a promising re-emerging control strategy, phages are environmentally sound since they are natural predators of, and specific only to, target bacteria. We isolated a jumbo phage and designated it Ralstonia phage RsoM2USA using our improved phage naming convention to reflect the phage's bacterial host species, phylogeny and geographic origin. Jumbo phage RsoM2USA belongs to the family *Myoviridae*, with particles having an icosahedral head of 142 nm in diameter, a long tail of 125 nm, a baseplate and tail fibers of 70 nm in length. The phage has a long latent period of 4.5 h and completes its infection cycle in 6 h with a burst size of approximately 32 particles per cell. With its genome size of 343,806 bp, RsoM2USA is the largest *Ralstonia*-infecting phage sequenced and reported to date. Out of the 486 ORFs annotated for RsoM2USA, only 80 could be assigned putative functions in replication, transcription, and translation including 44 tRNAs, as well as structure with the main structural proteins experimentally confirmed. Phylogenetically, RsoM2USA is most closely related to Xanthomonas phage XacN1, prompting a proposal of a new genus for the two jumbo phages. Jumbo phage RsoM2USA is a lytic phage and has a wide host range, infecting each of the three newly established *Ralstonia* species: *R. solanacearum*, *R. pseudosolanacearum*, and *R. syzygii*, and significantly reduced the virulence of its susceptible *R. solanacearum* strain in tomato plants. This suggests that RsoM2USA has the potential to be developed into an effective control against diseases caused by *R. solanacearum* species complex strains.

B12

Wilt disease suppression via rhizosphere microbiome transplant.

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Despite fecal microbiome transplantation has been used as a promising approach for human and animal health, the efficacy and potential implications of rhizosphere microbiome transplant (RMT) in plant disease management have only scarcely been explored. Here, we studied 6 resistant and 6 susceptible Solanaceae varieties to bacterial wilt disease caused by *Ralstonia solanacearum* in a 3-years field trial. We profiled the landscape rhizosphere microbiomes both in resistant and susceptible plant varieties and tested the feasibility of using RMT from the 6 resistant varieties to a susceptible model tomato variety Micro-Tom. We found the rhizo-microbiome of resistant varieties to enrich for specific bacterial taxa potentially associated with the wilt disease suppression. Quantification of the RMT efficacy using source tracking analysis revealed that more than 60% of the donor microbial communities successfully colonized the rhizosphere of recipient plants. RTM from resistant varieties resulted in different levels of wilt disease suppression, reaching up to 47% of reduction in disease incidence. Last, we provide a culture-dependent validation of potential bacterial taxa associated with antagonistic interactions with the pathogen, thus contributing to a better understanding of the potential mechanism associated with the disease suppression. Our study shows RMT as a promising tool to manipulate and engineer protective microbiomes to promote plant health, and advocate for future studies aiming at understanding the ecological mechanisms mediating the coalescence of donor and recipient microbiomes in the plant rhizosphere.

Travel Award

B12

Enhancement of synbiotics on microbial resistance against soil-borne *Ralstonia* disease.

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Improving the natural ability of the plant microbiome to prevent disease may help reduce our dependency on pesticides. However, the practical use of beneficial microorganisms is limited by three main constraints: Introduced microorganisms often fail to establish in the rhizosphere, do not express the desired function and during adaptation to the rhizosphere may lose the traits required for pathogen control. In this presentation I will highlight that these apparent constraints can be easily overcome by designing prebiotics treatments answering the ecological needs of the introduced microorganisms. Introduced microorganisms must compete with the native microbiome and secrete bioactive compounds. These requirements can be supported by using synbiotics, a mix of probiotic bacteria supported by prebiotic molecules. I will demonstrate that plant-derived prebiotic molecules can selectively improve the competitiveness and plant protective potential of the introduced beneficial bacteria. Used as a resource, prebiotics can create a niche for the introduced strain, thereby improving it's fitness. Further, by modulating bacterial physiology, they can activate traits needed for colonization (motility, biofilm formation) and even stimulate the production of bioactive molecules involved in plant protection. conclude by presenting a conceptual framework to match pre- and probiotic molecules for a more targeted and efficient plant protection and showing early success of this approach under field conditions.

B14

Gluconoacetobacter diazotrophicus promotes resistance to *Ralstonia pseudosolanacearum* inducing plant defense routes.

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Systemic resistance ISR is induced by some non-pathogenic bacteria that can suppress disease in plants. Our group has previously demonstrated *Gluconacetobacter diazotrophicus* (Gd) exerts a protective role against *Ralstonia pseudosolanacearum* (Rso) in *Solanum lycopersicum* cv. Rio Grande. Expression levels of ISR associated gene *pdf1.2*, defence related genes *pr1*, *pr5*, and *myc2*, and a *fitness*, related to plant redox status, were evaluated in *Arabidopsis thaliana* after inoculation with Gd, Rso, and when treated with both. Seeds of *A. thaliana* Col-0 were cultivated in a growth chamber under controlled conditions. After 14 days, plants were inoculated by soil drenching with 10^6 CFU/g of soil of *Gd* Pal5 (Gd+) or *Rso* GMI1000 (Rso+). Controls (C) were inoculated with sterile water. Double inoculated plants (Gd+Rso+) resulted of inoculating Gd+ plants with 10^6CFU/g of soil of *Rso* 5 days after the first inoculation. 5 days after each treatment, total RNA in aerial parts was isolated, cDNA synthetized and qPCR carried out using HOT FIREPol EvaGreen qPCR Mix Plus (Solis Biodyne), following the manufacturer's instructions. StepOne Real-Time PCR system (Applied Biosystems) was used. Three biological replicates were analysed three times. *Fitness* downregulation in Rso+ plants pointed out these plants were under ROS stress. Gd+ plants showed increased expression levels of PR1 than un-inoculated plants. Expression of this gene is greater in Gd+Rso+ plants than in those inoculated with one bacteria supporting priming as the process by which Gd prepares the plant for a subsequent pathogen attack. MYC encodes a transcriptional activator necessary for the response to JA and is repressed under all treatments. The endophytic beneficial bacterium produces a priming in defense routes of the plant that prevents pathogen colonisation, mediated by SA and not JA. Further studies of ISR related genes expression in roots and at different times post inoculation are needed.

B15

Reaction of chickpea cultivars to bacterial wilt, a new disease to a crop under expansion in Brazil.

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Chickpea (*Cicer arietinum* L.) is an annual grain legume used mainly as human food. It is well known as an inexpensive source of protein. In Brazil, chickpea's area increased from 800 ha in 2015 to 12,000 ha in 2019. The pathogen affects not only solanaceous plants, but also a wide host range. Since *R. pseudosolanacearum* was recently reported in chickpea in Brazil, the aim of this work is to provide information about resistance and susceptibility of the main chickpea cultivars cultivated in Brazil. An experiment was conducted including five chickpea cultivars (Cícero, Aleppo, Cristalino, Kalifa and Toro) and tomato – "Duradouro" cultivar (*Solanum lycopersicum*) as positive control and six *R. solanacearum* and *R. pseudosolanacearum* isolates. Each treatment consisted of sixteen plants sown in four 0.5 L plastic pots in three replicates. The seedlings were sprayed by a hand spray with a bacterial suspension calibrated to 10^8UFC/mL and kept in a greenhouse (20 and 40°C). The factorial experiment was carried out in a complete randomized design. Descriptive statistics was conducted evaluating the total number of symptomatic plants. All isolates wilted a large amount of tomato seedlings. Isolates GB (*R. pseudosolanacearum)*, originally from chickpea, showed a lesser capability of causing wilt in this host, suggesting a lack of co-evolution with this host. Others isolates, all *R. solanacearum* showed a higher virulence on chickpea, especially CNPH-RS476. Among chickpea cultivars, 'Cicero', the first chickpea cultivar available on Brazilian market, lacked resistance when compared to other cultivars tested. Kalifa, a recently released cultivar, was developed considering disease resistance, which was confirmed here with a lesser number of symptomatic plants, being the most recommended if the bacteria is present within the production area. This work also confirmed that not only Brazilian isolates of *R. pseudosolanacearum* but also *R. solanacearum* are capable of infecting chickpea cultivars.

B16

Grafting onto “baquicha” confers super protection on tomato against bacterial wilt.

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Bacterial wilt, caused by the soilborne bacteria *Ralstonia solanacearum* (race 1, biovar 1 and 2, phylotype II) and *R. pseudosolanacearum* (race 1, biovar 3, phylotype I), is highly destructive in tomato cultivated in under high temperatures and high soil moisture, common conditions in open field and under greenhouse. Cultivars available in Brazil are susceptible to bacterial wilt, but losses can be significantly reduced if they are grafted onto resistant tomato rootstocks, a technique well accepted especially among growers of greenhouse-grown high-value tomatoes. However, the protection offered by the available hybrid rootstocks is not stable and can be overcome in the presence of highly virulent variants of the pathogen, reported in Brazil and elsewhere. The objective of this study was to evaluate the reaction of the thornless *Solanum stramonifolium* access, known as “baquicha”, with four isolates of the *Ralstonia* species complex, RS 476 (race 1, biovar 3, phylotype I); RS 488 (Race 1, biovar 2, phylotype II); RS 594 (race 1, biovar 3, phylotype I) and RS 652 (race 1, biovar 1, phylotype II), previously selected for their geographical region and differential virulence on putative-resistant tomato rootstocks. The experiment was run with artificial root inoculation ($5x10^7$ ufc/mL) on 30-day old tomato seedlings, in three replicates of six plants each, under greenhouse conditions (20-40°C). “Baquicha” was compared with susceptible cultivars Kiara and Duradoro and with the resistant rootstock ‘Muralha’ (Takii Seeds), which, in previous experiment, ranked among the best for resistance when compared to other commercial hybrid rootstocks released as resistant to bacterial wilt. Seven days after inoculation, all plants of the susceptible cultivars were totally wilt, whereas plants of “baquicha” were symptomless, condition that persisted up to 20 days after inoculation. The resistance of ‘Muralha’ was effective only to isolate RS476, being overcome specially to isolate RS 652.

B17

Sustainable natural bioresources: coumarins mediate resistance against *Ralstonia solanacearum* in tobacco through jasmonic acid signaling.

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Plants deploy a variety of secondary metabolites to induce resistance, called plant elicitors, can protect plants from pathogens. Certain coumarins could be accumulated in response to plant pathogens invasion. However, the mechanism of coumarins involved in tobacco resistance against Ralstonia solanacearum remain unknown. To elucidate the tobacco transcriptome and metabolome response to R. solanacearum infection, leaves of tobacco cultivar K326 were infected with R. solanacearum for 0, 24, 48, 96 h. The combined results show that resistance to R. solanacearum in tobacco is associated with coumarins, phytohormones and other metabolic pathways. Several coumarins were enriched in tobacco plant during R. solanacearum infection. We found application of scopoletin (SC) and daphnetin (DA) could initiating systemic acquired resistance (SAR) in tobacco against plant pathogens invasion. Indeed, SC and DA significantly induced immune responses against tobacco bacterial wilt, including the accumulation of H2O2 and lignin, increases in defense enzymes and expression of pathogenesis-related (PR) proteins. In addition, accumulation of jasmonic acid (JA) in the leaves and upregulation of JA pathway genes confirmed that JA was involved in the defensive signals. Moreover, BAK1-silenced tobacco plants exhibited enhanced susceptibility to R. solanacearum supplemented with SC and DA. We demonstrated that application of coumarins in the field enhances tobacco growth and reducing the damage caused by R. solanacearum. Further, we show that silenced genes of the coumarins synthesis pathway in a resistance variety of tobacco enhanced susceptibility to R. solanacearum and improved disease progression. Collectively, this study unravels the use of coumarins in manipulating tobacco to generate defense responses against R. solanacearum, which might be attributed to JA-mediated induced resistance. The study reveals a mode of action by natural plant defense elicitor can suppress bacteria population and increase crop yield.

B18

Novel assay to detect *Ralstonia solanacearum* causing Moko disease in banana.

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Banana plants were domesticated in Southeast Asia and Melanesia. When brought for cultivation to Latin America, a new encounter disease called Moko emerged causing significant crop losses. Currently endemic in Latin America, this disease has spread to the Philippines and peninsular Malaysia. Causal agents of Moko are genetically diverse strains from the *Ralstonia solanacearum* species complex. Sequencing of different genomic regions led to the classification of Moko-causing strains into phylotype II and, within this phylotype, seven sequence variants (or sequevars) were defined based on 1% divergence of partial sequences of the endoglucanase-encoding gene *egl*. Two molecular assays for the diagnostics of Moko have been reported previously. One assay reported by Prior and Fegan in 2005 is comprised of a multiplex PCR that targets four sequevars. The other was reported in 2015 by Cellier *et al*. and consists of a duplex PCR that targets all seven sequevars and particular strains from sequevar 4 that are non-pathogenic to banana. To assess whether all sequevars of *R. solanacearum* associated with Moko can be detected by existing diagnostic methods, we validated these assays for analytical specificity (inclusivity and exclusivity), limit of detection, accuracy, and ruggedness. Our validations included DNA of recently collected strains of *R. solanacearum* from Costa Rica and Brazil, and DNA material from the *Ralstonia* collection of the Queensland Plant Pathology Herbarium in Australia. We tested 199 *Ralstonia* isolates, amongst which 106 are associated with Moko-infected banana. In addition, we included 32 endophytic bacterial isolates associated with healthy banana plants. Our results showed that neither of the published assays could detect isolates classified into three sequevars endemic to Brazil. The multiplex PCR published by Prior and Fegan reliably detected four sequevars originally targeted by this assay, but the duplex PCR developed by Cellier *et al.* did not detect approximately half the tested isolates associated with Moko-infected banana hosts. These results prompted us to design and validate two novel multiplex diagnostic assays based on conventional PCR that target the three sequevars endemic to Brazil. Our validation tests revealed that our newly developed assays are specific to these sequevars and sensitive. These diagnostic assays will increase preparedness for new incursions in banana-producing countries by providing means for early detection, subsequent containment, and control of Moko.

B19

Missions of the Plant Health Laboratory as a National Reference Laboratory regarding the detection of *Ralstonia solanacearum* species complex.

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The Plant Health Laboratory (LSV) is the National Reference Laboratory in charge of the official analysis of emerging and regulated quarantine pests in France. Two laboratories, one in Angers (BVO for Bacteriology, Virology and GMO detection) and one in Reunion Island (RAPT for Tropical Pests and Pathogens) have National Reference Laboratory mandates for bacterial plant pathogens, including *Ralstonia solanacearum, Ralstonia pseudosolanacearum, Ralstonia syzygii* species.
Different detection schemes were developed in both laboratories, based on the EPPO protocol PM7/21 (3) and the European Union Commission Implementing Regulation 2022/1193, for official analyses in the framework of third countries import or territory surveillance. To detect and identify this telluric bacterium, different official protocols have been defined on plant samples with or without symptoms (tubers, roots, stems), water (waste water and river) and soil. All these protocols lead to isolate strains, to identify the phylotypes and to verify the pathogenicity.
Since the first detection of *Ralstonia* in France in 1990's, the thousands of samples tested allowed to collect several hundreds of strains, stored in the internal collections of Plant Health Laboratory, or deposited at the CIRM-CFBP of Angers or at the 3P collection of Reunion Island. These strains are relevant and precious in order to address scientific questions about the epidemiological status of the disease. The strain collection from metropolitan France will be exploited in the framework of a PhD project started in 2022.

B20

Molecular differentiation of *Ralstonia solanacearum* biovars I, II and III.

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Ralstonia solanacearum, causal agent of the bacterial wilt, causes serious losses to several crops economically important. This bacterial species has been classified in five races (R) based on pathogenicity to different host plants: Race 1, the most widely distributed in the world, affects solanaceous and other host plants from several botanical families; Race 2 causes the Moko disease on bananas in South America, Philippines and Asia, and also infects Heliconia and ornamental Musaceae; Race 3 affects potato, tomato and Pelargonium in Brazil and United States; Race 4 infects ginger and occurs in Philippines; and Race 5 affects mulberry plants. However, its classification could be done at biovar (Bv) according to the utilization of sugars and hexose alcohols, where 5 levels have already been identified; and phylotype in 4 groups, based on the study of partial sequences of the spacer region 16S-23S rDNA (ITS), endoglucanase (*egl*) and *mutS* genes. Phylotype I includes strains from Asia belonging to Bvs III, IV and V; phylotype II, strains from America, classified in the Bvs I, II and IIT; phylotype III includes the strains from Africa Bvs I and IIT; and phylotypes IV strains from Indonesia Bvs I, II and IIT. The diagnosis at Bv level is laborious and time consuming as it involves tests of different sugars and alcohols utilization. This study aimed to investigate the resolving power of technique PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) of endoglucanase (*egl*) gene technique for classifying *Ralstonia solanacearum* strains at Bv level. DNAs from strains belonging I, II and III Bvs that occur in Brazil were amplified and digested with *HaeII* and *CfoI* restriction enzymes and the results showed distinct profiles for each one Bvs. Our new protocol offers a secure diagnosis of *Ralstonia solanacearum* strains at biovar level without the need of biochemical tests or *egl* gene sequencing.

B21

First report of *Ralstonia solanacearum* on *Kalanchoe blossfeldiana* in Brazil.

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Kalanchoe blossfeldiana Poelln. are herbaceous, succulent and erect plants with 20 to 30 cm tall originating from Madagascar. Cultivation should be carried without excess water in brightly lit areas and its propagation occurs mainly through seeds. Due to the fact that they have intense colorful flowering and durable for long time, these plants of the Crassulaceae family are widely used for ornamental purposes. In April 2018, *K. blossfeldiana* plants from Holambra city, São Paulo, Brazil were sent to Laboratório de Bacteriologia Vegetal, Instituto Biológico, with symptoms of widespread wilting and discoloration of xylem vessels. The optical microscope examinations showed intense bacterial flow and from there microorganisms were isolated. Moreover, plant material when submitted to the bacterial streaming test showed exudation characteristic of *Ralstonia solanacearum* specie. After incubation, growth of non-pigment and Gram-negative bacteria were observed in King's B medium with morphological characteristics similar to *Ralstonia* genus. (OEPP / EPPO. 2004). Subsequently, hypersensitivity reaction tests were performed on tobacco leaves with positive results in 24 hours. Artificial inoculations in tomato seedlings, made by pricking the leaflets stem with a needle immersed in bacterial suspension (10_8 CFU.mL^{-1}) reproduced the symptoms of wilting and chlorosis four days after inoculation, from where it was possible reisolate the bacteria. These results indicated that bacteria isolated from *K. blossfeldiana* belong to race I of *R. solanacearum* (JANSE, 1991). Molecular tests were performed to confirm the classification of the pathogen by the PCR-RFLP (*Restriction Fragment Length Polymorphism*) technique of endoglucanase gene (*egl*). Results using *Hae* III and *Cfo* I endonucleases confirmed the identification of *R. solanacearum* strains as belonging to biovar III. This is the first report of *R. solanacearum* occurring *K. blossfeldiana* in Brazil.

B22

Microbiome and transcriptome analysis of a bacterial wilt resistant tomato plant transplanted with two different soil microbiotas.

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Bacterial wilt (BW) caused by *Ralstonia solanacearum* greatly reduces the production of Solanaceae crops including tomato plant. BW resistance of tomato plant is known as quantitative trait, however, the underlying mechanism is largely unknown. Previously, BW-resistant tomato cultivar Hawaii 7996 (H6) transplanted with upland soil microbial fraction (UpMF) showed the strong resistance to BW, while the BW-resistance was completely abolished by transplant of forest soil microbial fraction (FoMF). In this study, we investigated the microbiotas of the rhizosphere of H6 tomato plant with UpMF and FoMF microbiota transplant and identified transplant MF-dependent microbial community in the rhizosphere. To compare differentially expressed genes (DEGs) between two different microbiota transplant, RNA-Seq was performed using UpMF and FoMF-transplanted H6 root samples. According to GO enrichment analysis of DEGs, the transplantation of UpMF in H6 up-regulated the expression of genes involved in diverse molecular signaling including plant cell-wall biosynthesis, compared to FoMF transplant. Based on the rhizosphere microbiome analysis of UpMF and FoMF transplanted H6, we construct the UpMF mimicking synthetic community (SynCom). Compared to control, treatment of UpMF mimicking SynCom induced BW resistance in H6, but not in MM. Taken together, these results suggest that the specific microbiota confers tomato plants with BW resistance in a cultivar-dependent manner. Further study using SynCom transplant is ongoing to analyze the expression pattern of genes involved in microbiota-specific signaling in tomato root.

B23

Phage therapy for biocontrol of bacterial wilt in strategic crops.

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Control of infections caused by *Ralstonia solanacearum* in plants, especially in strategic crops, is a challenging issue. Phage application can be considered as a potentially effective tool, supplementing existing methods, for the prevention of infection spread in the environment and in the seed plant material. This can be especially important for areas where the bacterium has been established recently and subsequent movement of the pathogen should be prevented.

In the present communication, we report on the isolation and selection of bacteriophages active to *R. solanacearum* (Rs phages) for further application in strain subtyping and infection control strategy. Twenty-five phages were isolated from environmental samples on 40 Georgian isolates of *R. solanacearum* and characterized by main biological properties. The prevalence of Myoviridae-type morphology among *Rs* phages was shown although single phages appeared to be Podoviruses and Siphoviruses. Four selected Rs phages were included in the experimental mixture and their antibacterial efficacy was examined in the lab conditions on potato tuber discs, also *in vivo* challenge experiments on growing potato plants. A promising probability of phage application on seed material and young plants was shown. The phage treatment resulted in lowered numbers of diseased plants and delays in disease development, that was depending on the treatment regimen.

The efficacy and reliability of phage-based biocontrol of bacterial wilt in plants can be further improved by: i) increasing coverage of possible varieties of target pathogens - through regular screenings on emerging strains and enrichment of phage mixtures with different lytic phages; ii) applying phage mixtures with broad-host-range adapted phages to minimize the development of phage resistance in bacteria; iii) increasing environmental stability of phage preparations by employing protective formulations, avoiding sunlight, high acidity etc.

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