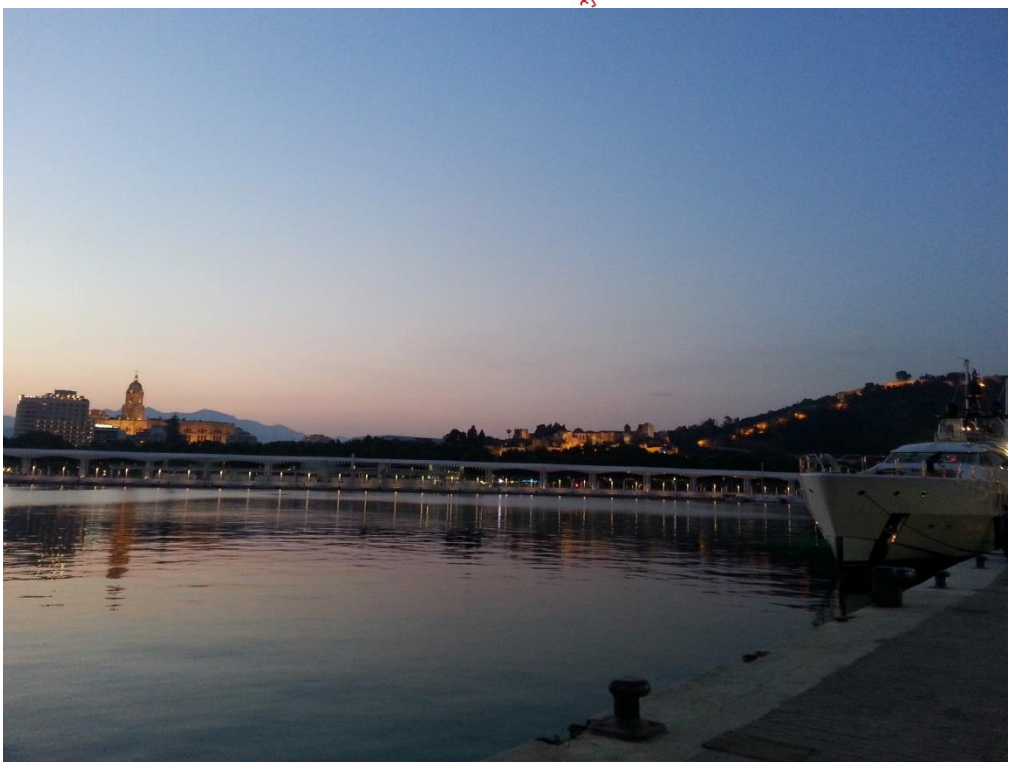
9th International Conference on *Pseudomonas syringae* and Related Pathogens

Málaga, 2nd-5th June 2015



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The 9th International Conference on *Pseudomonas syringae* pathovars and related pathogens.

June 2nd – June 5th 2015

Málaga, Spain.

Dear Delegate,
Welcome to the 9th International Conference on *Pseudomonas syringae* pathovars and related pathogens 2015 meeting in Málaga. The organizing committee hopes that you have a pleasant and productive time at the meeting. This booklet contains the programme and abstracts.

Maps
A Map of Málaga city is included in the delegate pack.

Accommodation
The conference sessions will be held at the NH hotel of Malaga. Location and arrival details can be found at the Hotel's web page. Although Malaga has a very good net for visitor's accommodation, early June is in the top-touristic season, so we suggest you to book your rooms as soon as possible. The NH Hotel is obviously a convenient place to stay while attending the conference, but there are several optional hotels, some of which are suggested in the Accommodation section of our web site. For instance, the hotels Novotel and IBIS Budget are only 50 meters away from the NH, while the hotels Molina Lario and Malaga Palacio are about 800 meters (10 min walk).

Registration and Information
The congress fee includes the participation in the congress, the congress materials, lunch on Wednesday, Thursday and Friday, coffee breaks, welcome reception, social programme and gala dinner.
Registration deadline: 6th June, 2015

Principal Investigator:	Up to 30/04/2015: 450€; After 01/05/2015: 500€
Postdoctoral delegates:	Up to 30/04/2015: 450€; After 01/05/2015: 500€
Student delegates:	Up to 30/04/2015: 375€; After 01/05/2015: 425€
Industry:	Up to 30/04/2015: 450€; After 01/05/2015: 500€

Welcome reception
A welcome lecture will take place on Tuesday evening from 19.30 in the main building at the University of Málaga (C/ Paseo del Parque, 2), followed by a drinks reception in the terrace.

Talks
Long (25 + 5 minutes) and short (12 + 3 minutes) Talks will be in the NH Hotel of Málaga. Speakers should upload their Powerpoint presentation well in advance of their allocated time, and no later than the break immediately preceding the relevant session. Slide preview facilities are available, please ask for details if required.

Posters
Poster sessions will be held closer to the coffee and tea place. Your poster number can be found in the abstract booklet.
Session posters should be displayed from 09.30 Wednesday until 19.30 Thursday.
Special session for poster would be allocated on Thursday, from 13.30 to 17.00 (including coffee and tea time).

Lunches and Dinners

Lunches on Wednesday to Friday will be provided in the dining hall at NH Málaga Hotel. The Conference Dinner on Friday will be by ticket only and will take place in "Los Patios de Beatas" (http://lospatiosdebeatas.com/)**.**

The football club and informal social event includes a light dinner which will take place at the beach from 20.00-late on Thursday.

Internet Access

Wireless internet access is available at the NH Málaga Hotel. Wi-Fi is also available at numerous coffee shops and cafes throughout Málaga.

Local organizing committee

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We would like to thank the following sponsors for supporting this meeting:

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Tuesday 2nd June		Wednesday 3rd June		Thursday 4th June		Friday 5th June	
		09:00 (30 min)	Session I: Epidemiology and Disease Control	09:00 (30 min)	Session V: Genomics and Bioinformatics		
		09:30 (30 min)		09:30 (30 min)		09:30 (30 min)	COST-SUSTAIN Session on effectors and plant responses
		10:00 (15 min)		10:00 (15 min)		10:00 (30 min)	
		10:15 (15 min)		10:15 (15 min)			
		10:30 (15 min)		10:30 (15 min)		10:30 (30 min)	
		10:45 (15 min)					
		11:00 coffee		11:00 coffee		11:00 coffee	
		11:30 (20 min)	Session II: Pathogenesis	11:30 (30 min)	Sessión VI: Emerging Research Areas	11:30 (30 min)	COST SUSTAIN Session on effectors and plant responses
		11:50 (20 min)		12:00 (15 min)		12:00 (30 min)	
		12:10 (15 min)		12:15 (15 min)		12:30 (30 min)	
		12:25 (15 min)		12:30 (15 min)			
		12:40 (15 min)		12:45 (15 min)			
		12:55 (15 min)				13:15 13-45	Closing ceremony
		13:10 (15 min)					
		13:30 Lunch		13:30 Lunch			
		15:00 (30 min)	Session III: Ecology	15:00 (90 min)	Poster Session	14:00 Lunch	
16:00-18:00	Registration (NH Hotel Málaga) Acreditation will continue at the University Building	15:30 (30 min)					
		16:00 (15 min)					
		16:15 (15 min)					
		16.30 Tea		16:30 Tea			
		17:00 (30 min)	Session IV: Effectors and Plant Responses	17:00 (30 min)	Session VII: Evolution, Taxonomy and Systematics		
		17:30 (30 min)		17:30 (30 min)			
		18:00 (15 min)		18:00 (15 min)			
		18:15 (15 min)		18:15 (15 min)			
		18:30 (15 min)		18:30 (15 min)			
		18:45 (15 min)					
19.15 (15 min)	Opening (UMA Government Building)						
19:30 (30 min):	Welcome lectura (UMA Government Building)			20.00-23.30	FOOTBALL CLUB AND INFORMAL SOCIAL EVENT		
20.00-23.00	RECEPTION (UMA Government Building)	20.30-22.00	MALAGA CITY TOUR			21.30	CONFERENCE DINNER

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Programme

SCIENTIFIC PROGRAM

Tuesday, 2nd June

16.30-18.00 Registration (VENUE: NH Hotel Málaga). Acreditation will continue at the University Building.

19.15-19.30 Opening (University Main Government Building "Rectorado", Av. Cervantes nº2)

19.30-20.00 Welcome lecture. Dr. Jesus Murillo (Universidad Pública de Navarra, Spain)

20.00-23.00 Reception (University Main Government Building "Rectorado", Av. Cervantes nº2)

Wednesday, 3rd June

9.00-11.00 Session I: Epidemiology and disease control.

Chairs: Dr. Boris Vitnatzer and Dr. Honour McCann.

9.00-9.30 Plenary talk. Dr. Boris Vinatzer. (Virginia Tech, USA) p. 13

"Genome similarity-based codes to precisely classify and name pathogens that cause emerging infectious diseases of humans, animals and plants."

9.30-10.00 Plenary talk. Dr. Honour MaCann (Massey University, New Zealand) p.14

"*Evolution and population genomics of Pseudomonas syringae pv. actinidiae* "

10.00-10.15 Short talk. Dr. Stefania Tegli (University of Florence, Italy) p.15

"*Virulence inhibiting peptides for the environmentally friendly control of plant diseases caused by Pseudomonas syringae*"

10.15-10.30 Short talk. Dr. Joel Vanneste (Ruakura Research Centre, New Zealand) p.16

"*Recent development in control options for bacterial canker of kiwifruit caused by Pseudomonas syringae pv. syringae*"

10.30-10.45 Short talk. Dr. David Baltrus (University of Arizona, USA) p.17

"*A programmable, selective, phage-derived bacteriocin locus conserved across Pseudomonas syringae*"

10,45-11.00 Short talk. Dr. Amandine Cunty (INRA, France) p.18

"*Multilocus VNTR analysis of P. s. pv. actinidae and pv. actinidifoliorum strains isolated from symptomatic kiwifruit*"

11.00-11.30 Coffee

11.30-13-30 Session II: Pathogenesis.

Chairs: Dr. Cayo Ramos and Dr. Yuki Ichinose.

11.30-11.50 Plenary talk. Dr. Cayo Ramos (Universidad de Málaga, Spain) p.21

"*Novel Pseudomonas savastanoi pv. savastanoi virulence factors and their contribution to knot formation in olive plants*"

11.50-12.10 Plenary talk. Dr. Yuki Ichinose (Okayama University, Japan) p.22

"*Global regulation of virulence-related genes in Pseudomonas syringae pv. tabaci*"

12.10-12.25 Short talk. Dr. Mari Trini Gallegos (E. E. "El Zaidín-CSIC, Spain) p.23

"*Flagella-independent motility of Pseudomonas syringae pv. tomato DC3000 and its contribution to virulence towards tomato plants*"

12.25-12.40 Short talk. Dr. Tally Rosenberg (The Hebrew University of Jerusalem, Israel) p.24

"*Involvement of type IV pili in Acidovorax citrulli virulence*"

12.40-12.55 Short talk. Dra. Emilia López-Solanilla (Universidad Politécnica de Madrid, Spain) p.25

"*Perception of plant and environmental signals in Pseudomonas syringae pv. tomato DC3000*"

12.55-13-10 Short talk. Dr. Milija Jovanovic (Imperial College London, UK) p.26

"*Functional characterization of the residues in regulatory proteins hrpg and hrpv of Pseudomonas syringae pv tomato DC3000*"

13.10-13-25 Short talk. Dr. Hai-Lei Wei (Cornell University, USA) p.27

"*Pseudomonas syringae effectorless polymutants reveal novel interplay between effectors*"

13.30-15.00 Lunch

15.00-16.30 Session III: Ecology.

Chairs: Dr. Cindy Morris and Dr. Marco Scortichini.

15.00-15.30 Plenary talk. Dr. Cindy Morris (INRA, France) p.31

"*From crops to clouds and back again: the evolving story of a bacterium sans frontier*"

15.30-16.00 Plenary talk. Dr. Marco Scortichini (CRA, Italy) p.32

"*Beyond the symptom: the lifestyle of plant pathogenic pseudomonads when they are not inciting disease*"

16.00-16.15 Short talk. Dr. Oddur Vilhelmsson (University of Akureyri, Iceland) p.33

"*Are lichens natural reservoirs for plant pathogens?*"

16.15-16.30 Short talk. Dr. Olile Berge (INRA, France) p.34

"*A broad and operational classification framework to address the ecology of Pseudomonas syringae populations*"

16.30-17.00 Tea

17.00-19.00 Session IV: Effectors and Plant Responses.

Chairs: Dr. James Alfano and Dr. Joana Jelenska.

17.00-17.30 Plenary talk. Dr. James Alfano (University of Nebraska, USA) p.37

"*Pseudomonas syringae type III effectors, their targets, and suppression of plant immunity*"

17.30-18.00 Plenary talk. Dr. Joana Jelenska (The University of Chicago, USA) p.38

"*Fate of P. syringae molecules in plants*"

18.00-18.15 Short talk. Dr. Ofir Bahar (Volcani Center, Israel) p.39

"*Outer membrane vesicles are potent inducers of plant immunity*"

18.15-18.30 Short talk. Dr. Saul Burdman (The Hebrew University of Jerusalem, Israel) p.40

"*Genetic and functional characterization of Acidovorax citrulli type III effectors*"

18.30-18.45 Short talk. Dr. Christopher Waite (Imperial College, UK) p.41

"*Roles for the sensor-kinase RetS, small RNAs and divergent RsmA protein in T3SS regulation*"

18.45-19.00 Short talk. Dr. Darell Desveaux (University of Toronto, Canada) p.42

"*Functions of the HopZ type III effector family in Arabidopsis*"

Thursday, 4th June

9.00-11.00 Session V: Genomics and Bioinformatics.

Chairs: Dr. Charles Manceau and Dr. Rob Jackson

9.00-9.30 Plenary talk. Dr. Charles Manceau (Anses, France) p.45

"*Phylogenetic and VNTR analysis identified non-pathogenic lineages within Xanthomonas arboricola associated with walnut lacking the canonical type three secretion system*"

9.30-10.00 Plenary talk. Dr. Rob Jackson (University of Reading, UK) p.46

"*Regulatory innovation rescues a catastrophic motility defect - an insight to bacterial robustness and resilience for a plant growth-promoting bacterium?*"

10.00-10.15 Short talk. Dr. Noam Eckshtain-Levi (The Hebrew University of Jerusalem, Israel) p.47

"*Does bigger mean better? First lessons from comparative genomics in Acidovorax citrulli*"

10.15-10.30 Short talk. Dr. Marina Puigvert (Universitat de Barcelona, Spain) p.48

"*Transcriptome of the newly-sequenced Ralstonia solanacearum strain UY031 infecting the wild potato Solanum commersonii*"

10.30-10-45 Short talk. Dr. José A. Gutiérrez-Barranquero (IHSM-UMA-CSIC, Spain) p.49

"*Complete sequence and comparative genomic analysis of eight native Pseudomonas syringae plasmids belonging to the pPT23A family*"

11.00-11.30 Coffee

11.30-13.00 Session VI Emerging Research Areas.

Chairs: Dr. Gail Preston and Dr. Matthias Ullrich

11.30-12.00 Plenary talk. Dr. Gail Preston (University of Oxford, UK) p.53

"*The apoplastic arena in defence and disease*"

12.00-12.15 Short talk. Dr. Khaled Abdallah (Jacobs University, Germany) p.54

"*Molecular analysis of the role of LSCR, a small regulatory protein involved in expression of*

levansucrase in Pseudomonas syringae pv. glycinea PG4180"

12.15-12.30 Short talk. Dr. Reut Shavit (TheHebrew University of Jerusalem, Israel) p.55

"*A VAPBC toxin-antitoxin module is involved in stress response of the plant pathogen Acidovorax citrulli*"

12.30-12.45 Short talk. Dr. Chiaraluce Moretti (University of Perugia, Italy) p.56

"*Ca^{2+} transport in Pseudomonas savastanoi pv. savastanoi, the causal agent of olive knot disease*"

12.45-13.00 Short talk. Dr. Renier A.L. van der Hoorn (University of Oxford, UK) p.57

"*Manipulation of apoplastic hydrolases by Pseudomonas syringae*"

13.30-15.00 Lunch

15.00-16.30 Poster viewing

16.30-17.00 Tea

17.00-18.45 Session VII: Evolution, Taxonomy and Systematics.

Chairs: Dr. Dawn Arnold and Dr. David Guttman.

17.00-17.30 Plenary talk. Dr. Dawn Arnold (The University of the West of England, UK). P.61

"*A persistent reservoir of a genomic island in Pseudomonas syringae pv. phaseolicola*"

17.30-18.00 Plenary talk. Dr. David Guttman (University of Toronto). P.62

"*Comparative and evolutionary genomics of the Pseudomonas syringae species complex*"

18.00-18.15 Short talk. D. Michelle T. Hulin (East Malling Research, UK). P.63

"*Exploring the genetic basis of host specificity in Pseudomonas syringae*"

18.15-18.30 Short talk. Dra. María M. López (IVIA, Spain) p.64

"*An integrated approach of diagnostic to detect inter and intraspecific diversity in Erwinia, Xanthomonas and Pseudomonas*"

18.30-18.45 Short talk. Dra. Eva Arrebola (IHSM-UMA-CSIC, Spain) p.65

"*Relevant features to epiphytic and pathogenic lifestyles from the genome sequence of Pseudomonas syringae UMAF0158*"

Friday, 5th June

9.30-11.00 COST-SUSTAIN Session on Effectors and Plant Responses I. Chairs Carmen Beuzon and Nemo Peeters

9.30-10.00 Talk 1. Dr. Alberto Macho (Shangai Center for Plant Stress Biology, China) p.69

"*Bacterial type-III effectors and the suppression of plant immune signalling*"

10.00-10.30 Talk 2 Dr. Magda Krzymowska (Institute of Biochemistry and Biophysics, Poland) p.70

"*Cellular dynamics of HopQ1, a type III secretion effector from Pseudomonas syringae*"

10.30-11.00 Talk 3. Dr. Vardis Ntoukakis (University of Warwick, UK) p.71

"*Investigating the role of chromatin remodeling in plant immunity*"

11.00-11.30 Coffee

11.30-13.00 COST-SUSTAIN Session on Effectors and Plant Responses II.

11.30-12.00 Talk 4. Dr. Justin Lee (Leibniz Institute of Plant Biochemistry, Germany) p.72

"*A bacterial effector that specifically suppressed MAMP-induced activation of the MAPKs, MPK4 and MPK11*"

12.00-12.30 Talk 5. DR. Jens Boch (Martin Luther University, Germany) p.73

"*Natural diversity and functional requirements of TALEs in the Xanthomonas oryzae - rice interactions*"

12.30-13.00 Talk 6. Dra. Carmen R. Beuzón (IHSM-UMA-CSIC, Spain) p.74

"*Phenotypic heterogeneity and effector-mediated mechanisms of plant defence evasion in Pseudomonas syringae*"

13.15-13.45 Closing ceremony

14.00-16.00 Lunch

21.30 Conference Dinner

ABSTRACTS

ORAL PRESENTATIONS

SESSION I

EPIDEMIOLOGY AND DISEASE CONTROL

Genome similarity-based codes to precisely classify and name pathogens that cause emerging infectious diseases of humans, animals and plants.

Boris A. Vinatzer[1], Alexandra J. Weisberg[1], Caroline L. Monteil[1], Lenwood S. Heath[2]

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[2]Computer Science Department, Virginia Tech, Blacksburg, VA 24061, USA

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When a new human, animal, or plant disease emerges, it is often difficult to rapidly identify, classify, and name the new pathogen. A telling example was the emergence of bacterial canker of kiwifruit in 2008 in Italy and in the following years in other countries. Many different names have been proposed since then for the *Pseudomonas syringae* outbreak strain responsible for this epidemic. The community will at some point settle on one name. However, it would have been much easier if one permanent designation could have been assigned immediately at the dawn of the epidemic. Next generation genome sequencing is now fast and cheap enough to allow sequencing the genome of any new pathogen within weeks or days of an outbreak. Bioinformatic pipelines are now available to almost immediately compare the genome sequence of the new pathogen with genome sequences of similar already known pathogens. What is still missing is the translation of the calculated genome sequence similarity into meaningful strain names. We have thus developed the concept of Life Identification NumbersTM (LINsTM) that can be assigned to any genome-sequenced pathogen or any other organism or virus. Using the examples of *P. syringae* and ebolavirus genomes we show here how LINs can be sequentially assigned to emerging pathogens, how LINs precisely reflect genome similarity and phylogenetic relationships, and how LINs can be used as the basis for the description of named bacterial taxa.

Key Words: emerging diseases, genome sequencing, nomenclature, kiwifruit, ebolavirus.

Evolution and population genomics of *Pseudomonas syringae* pv. *actinidiae*

Honour C. McCann*[1], Li Li*[2], Yifei Liu[3], Matthew D. Templeton[4], Paul B. Rainey[1,5], Hongwen Huang**[3]**

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[2]Wuhan Botanical Garden, Chinese Academy of Sciences, China
[3]South China Botanical Garden, Chinese Academy of Sciences, China
[4]Institute for Plant and Food Research, NZ
[5]Max Planck Institute for Evolutionary Biology, Germany
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Our previous work predicted the existence of a genetically diverse and recombining source population of *Pseudomonas syringae* pv. *actinidiae* (*Psa*), from which stochastic sampling events followed by selection in agricultural environments for host specialization occurred. Sequencing an additional set of Chinese *Psa* isolates revealed far greater diversity than all other outbreak strains, suggesting the epidemic strain may have emerged from China and has been circulating there for longer. Since the recently domesticated *Actinidia* (kiwifruit) is native to China, it is possible that *Psa* has an ancestral association with wild kiwifruit. In order to identify the location of and extent of diversity within the source population of *Psa* and elucidate the evolutionary processes leading to its emergence, a population genomics study was initiated to sample *P. syringae* from kiwifruit across six provinces in China. In order to account for the possibility that *Psa* may have emerged from a non-agricultural source population associated with wild host populations, the sampling strategy therefore included both cultivated and wild kiwifruit plants. Sample sites within each province were chosen based either on the abundance of wild kiwifruit taxa (including close relatives of domesticated species) or their proximity to current or historically infected orchards. The presence of visible symptoms of disease was recorded and multiple Pseudomonads were recovered from each plant at each location. 833 of 1,459 total plant isolates have been genotyped and a subset of phylogeographically diverse isolates selected for whole genome sequencing.

Virulence inhibiting peptides for the environmentally friendly control of plant diseases caused by *Pseudomonas syringae*

Stefania Tegli[1], Matteo Cerboneschi[1], Carola Biancalani[1], Sofia Macconi[1], Francesco Tadini-Buoninsegni[2], Alessio Sacconi[3], Serena Smeazzetto[2], Maria Rosa Moncelli[2], Patrizia Bogani[4], Stefano Biricolti[5]

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The control and management of bacterial diseases of plants still rely mainly on applications of copper and antibiotics. In EU Member States, antibiotics are not allowed for plant protection, while copper is among the very few chemicals still authorised also in organic agriculture. However, its use was recently strictly regulated in EU for its ecotoxicological negative effects and for its impact on the increase of antibiotic-resistant bacteria into agroecosystems, with risks for human and animal health. A promising alternative to cop-per was proposed against *Erwinia amylovora* (Yang et al., 2014), but no sustainable options are available yet for the control of plant pathogenic bacteria belonging to the *Pseudomonas syringae* group. In this work we propose an innovative strategy, based on the design and use of peptides targeting the translocation of bacterial pathogenicity and virulence effectors by the Type Three Secretion System (T3SS), highly conserved and essential for the pathogenicity of Gram-negative bacteria, both of plants and of mammalian hosts including humans (Chatterjee et al., 2013). As a result of their distinguishing hall-mark, these virulence inhibiting peptides (VIPs) compromise the T3SS injection of T3 effectors into plant cells, instead of bacterial viability, thus to avoid or decrease the risk to develop any VIPs resistance. Using *P. savastanoi*, *P. syringae* pv. *tabaci* and *P. syringae* pv. *actinidiae* as model systems, VIPs were demonstrated to compromise in vitro and in vivo bacterial pathogenicity on hosts, and HR on Tobacco. No negative side-effects on model membranes and Ca-ATPase were found. VIPs-induced inhibition of T3SS assembly was confirmed by autoagglutination and Congo Red assays. The interaction between VIPs and their T3SS target was investigated by mutagenesis, and by combining electrochemical impedance spectroscopy and surface plasmon resonance measurements. VIPs effectiveness was also demonstrated by VIPs transient expression in *Nicotiana tabacum* challenged by *P. syringae* pv. *tabaci*.

Key Words: T3SS, virulence-inhibitors, oligopeptides, copper resistance, *P. syringae*.

REFERENCES

YANG, F.; KORBAN, S.S.; PUSEY, P.L.; ELOFSSON, M.; SUNDIN, G.W.; ZHAO, Y. (2014): Small-molecule inhibitors suppress the expression of both type III secretion and amylovoran biosynthesis genes in *Erwinia amylovora*. Molecular Plant Pathology, 15 (1), 44-57.
CHATTERJEE, S.; CHAUDHURY, S.; MCSHAN, A.C.; KAUR, K.; DE GUZMAN, R.N. (2013): Structure and biophysics of type III secretion in bacteria. Biochemistry, 52 (15), 2508-2517.

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Recent development in control options for bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae*

Joel L. Vanneste, Janet Yu, Deirdre A. Cornish, Luca Fiorentini, Francesco Spinelli, Tony Reglinski, Kirstin Wurms

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Following the discovery of *Pseudomonas syringae* pv. *actinidiae* (*Psa*) in New Zealand, a screening programme of over 350 products was conducted on young kiwifruit plants in a glasshouse in the hope of finding new compounds for the control of this pathogen. The screening programme was directed not only at compounds already known to kill or inhibit plant pathogenic bacteria, such as antibiotics or heavy metals (mostly copper), but also at biological control agents, bacteriophages, antimicrobial peptides, novel chemicals and elicitors. Under our experimental conditions, only a few of the products reduced the incidence of *Psa* significantly and consistently. Some of the reasons for this very low rate of success are linked with the epidemiology of the pathogen while others are linked with the characteristics of the products. Those reasons and the limitations associated with each type of control methods will be presented. Nevertheless, from this programme three products were subsequently registered in New Zealand for control of *Psa*, including an elicitor of the salicylic pathway. Green-fleshed kiwifruit (*Actinidia deliciosa*) and yellow-fleshed kiwifruit (*A. chinensis*) reacted differently to some elicitors. To understand the molecular mechanisms involved in the elicitation of resistance by kiwifruit plants, gene expression is being studied in *A. chinensis* 'Zesy002' (commonly known as Gold3) treated with different elicitor compounds before inoculation with Psa. Different tissues were sampled from 3 hours to 72 hours post-infection. A few genes whose expression increases after treatment have already been identified by qPCR. Some of these genes, including at least one involved in resistance to fungal pathogens and insects, might be used as potential markers of resistance in *A. chinensis*.

Key Words: Elicitors, SA/JA pathways, Gene expression, antibiotics, antimicrobial com-pounds.

A programmable, selective, phage-derived bacteriocin locus conserved across *Pseudomonas syringae*

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We have screened a phylogenetically diverse suite of *Pseudomonas syringae* strains for intra-specific killing activity. Killing activity was maintained, even after deletion of all identified bacteriocins from *P. syringae* pv. *syringae* B728a. Follow up genetic and genomic experiments demonstrate that *P. syringae* and related species contain a previously unrecognized, phage derived bacteriocin locus. This locus is similar in function to the R-type pyocins of *P. aeruginosa* but is independently evolved from a progenitor phage. This bacteriocin is capable of targeting and killing cells in a strain specific manner. Moreover, even though the whole bacteriocin locus contains >20 genes, we have successfully retargeted *P. syringae* B728a killing activity through the movement of only two genes into this strain. These two genes experience, and not other structural genes of this bacteriocin locus, experience high levels of horizontal transfer within *P. syringae* and across pseudomonad species and thus may be an important target for natural selection in nature. We further find that some *P. fluorescens* strains appear to be able to target and kill *P. syringae* using this same phage derived system.
Therefore, many *P. syringae* strains contain an active phage-derived bacteriocin locus which can be used to selectively kill closely related cells under natural conditions. Given that similar systems have been exploited as selective antimicrobials within *P. aeruginosa*, this *P. syringae* system may provide a unique platform for the development of next generation agricultural antimicrobials.

Key Words: bacteriocins, programmable antimicrobials, horizontal gene transfer, prophage,

Multilocus VNTR Analysis of *Pseudomonas syringae* pv. *actinidiae* and pv. *actinidifoliorum* strains isolated from symptomatic kiwifruit

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Pseudomonas syringae pv. *actinidiae* (*Psa*), the causal agent of bacterial canker on kiwifruit, was first detected in Europe in Italy in 2008 and later on in France in 2010. Over the last 5 years, 300 strains were isolated and characterized using phenotypic, pathogenic, PCR-based tests and Multilocus Sequence Analysis (MLSA) based on four housekeeping genes (*gapA*, *gltA*, *gyrB* and *rpoD*). Strains causing leaf spots, die-back, and canker were identified as *Psa* biovar 3 and strains causing only leaf spots were identified as *Pseudomonas syringae* pv. *actinidifoliorum* (*Psaf*) (Cunty et al., 2014). In order to track the spread and the origin of the epidemics of these two pathogens in France, we developed a Multilocus Variable-Number of Tantem Repeats (VNTR) Analysis (MLVA). The genomic sequences of *Psa* biovar 3 CFBP 7286 and *Psaf* ICMP 18807 were analyzed for potential tandem repeats. Eleven polymorphic VNTR loci were selected and used to type 340 strains of Psa biovar 3 isolated in Chile, China, France, Italy and New Zealand, and on 39 strains of *Psaf* isolated in Australia, France and New Zealand. Fifty-five and 16 MLVA types were identified within *Psa* biovar 3 and *Psaf* strains, respectively. MLVA and Discriminant Analysis in Principal Components revealed that strains isolated in Chile, China and New Zealand are genetically distinct from strains isolated in Italy and France, which appeared to be genetically closely related. In contrast, no structuring according to the place of isolation was observed for *Psaf*. This indicates that *Psa* biovar 3 in France may share the same origin than *Psa* biovar 3 strains isolated in Italy and that *Psaf* isolated in France are similar to those isolated in Australia and New Zealand. We developed the first MLVA scheme useful to explore the diversity and to trace the worldwide dispersal routes of *Psa* and *Psaf*.

Key Words: bacterial canker, MLSA, MLVA, DAPC.

REFERENCES

CUNTY, A.; POLIAKOFF F.; RIVOAL C.; CESBRON S.; FISCHER-LE SAUX M.; LEMAIRE C.; JACQUES MA.; MANCEAU C.; VANNESTE J. (2014): Characterization of *Pseudomonas syringae* pv. *actinidiae* (*Psa*) isolated from France and assignment of *Psa* biovar 4 to a de novo pathovar: *Pseudomonas syringae* pv. *actinidifoliorum* pv. nov. Plant Pathology, DOI: 10.1111/ppa.12297.

SESSION II

PATHOGENESIS

Novel *Pseudomonas savastanoi* pv. *savastanoi* virulence factors and their contribution to knot formation in olive plants

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Pseudomonas savastanoi pv. *savastanoi* (*Psv*), causative agent of olive (*Olea europaea*) knot disease, is an untraditional member of the *Pseusomonas syringae* complex, inducing aerial tumours instead of the foliar necroses and cankers characteristic of most members of this bacterial complex. Early investigations into virulence factors associated to Psv infections established that the phytohormones indole-3-acetic acid (IAA) and cytokinins, as well as the type-III secretion system (T3SS) and the quorum sensing regulatory system, are involved in knot development. At the previous "*P. syringae* and related pathogens" conference in Oxford (2010), we presented the annotation of the *Psv* NCPPB 3335 draft genome and the identification of novel virulence factors and several metabolic pathways required for full fitness of this pathogen in olive knots. During the last five years, our work focused in the functional analysis of several of these virulence determinants. We have demonstrated the delivery of eleven *Psv* NCPPB 3335 T3SS effectors into plant cells, including three proteins from two novel families of the *P. syringae* complex effector super-repertoire (HopBK and HopBL), one of which comprises two proteins (HopBL1 and HopBL2) that harbour a SUMO protease domain. We have also determined that two enzymes involved in the metabolism of the bacterial second messenger cyclic diguanylate-GMP (c-di-GMP), i.e. the phosphodiesterase BifA and the diguanylate cyclase DgcP, are required for full virulence of the bacterium in olive knots. Other characterized virulence determinants include a second iaaMH operon involved in the biosynthesis of IAA, as well as several operons encoded in a genomic region (about 15 Kb) of the *Psv* NCPPB 3335 chromosome, named VR8, that is absent in all sequenced *P. syringae* strains infecting herbaceous plants (non-lignified), but it is shared with other *P. syringae* pathovars infecting woody hosts (lignified).

Key Words: *Pseudomonas savastanoi*, T3SS, Cyclic-di-GMP, Polyphenols, Auxins.

REFERENCES

RAMOS, C.; MATAS, I.M.; BARDAJI, L.; ARAGÓN, I.M.; MURILLO2, J. (2012): *Pseudomonas savastanoi* pv. *savastanoi*: some like it knot. Molecular Plant Pathology, 13(9): 998-1009.
MATAS, I.M.; LAMBERTSEN, L.; RODRÍGUEZ-MORENO, L.; RAMOS, C. (2012): Identification of novel virulence genes and metabolic pathways required for full fitness of *Pseudomonas savastanoi* pv. *savastanoi* in olive knots. New Phytologist, 196: 1182-1196.
MATAS, I.M.; CASTAÑEDA-OJEDA, M.P.; ARAGÓN, I.M.; ANTÚNEZ-LAMAS, M.; MURILLO, J.; RODRÍGUEZ-PALENZUELA, P.; LÓPEZ-SOLANILLA, E.; RAMOS, C. (2014): Translocation and Functional Analysis of *Pseudomonas savastanoi* pv. *savastanoi* NCPPB 3335 Type III Secretion System Effectors Reveals Two Novel Effector Families of the *Pseudomonas syringae* Complex. Molecular Plant-Microbe Interactions, 27(5): 424-436.
ARAGON, I.M.; PÉREZ-MENDOZA, D.; MOSCOSO, J.A.; FAURE, E.; GUERY, B.; GALLEGOS, M.T.; FILLOUX, A.; RAMOS, C. (in press): Diguanylate cyclase DgcP is involved in plant and human *Pseudomonas* spp. Infections. Environmental Microbiology, doi: 10.1111/1462-2920.12856.

Global regulation of virulence-related genes in *Pseudomonas syringae* pv. *tabaci*

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Reverse genetics approach is a powerful method to study plant-microbe interactions. We have investigated Pseudomonas-plant interactions using a large variety of virulence-related mutants. Interestingly, the mutant often alters its gene expression profile. Flagellin, a major component of flagellum, possesses a well known microbe-associated molecular pattern, flg22, and induces plant defense (Felix et al. 1999). The Δ*fliC*, a flagellin-defective mutant, in *P. syringae* pv. *tabaci* lost flagellum and flagellar-mediated motility, and reduced the ability to cause disease symptoms (Ichinose et al. 2003). Interestingly, microarray analysis revealed that Δ*fliC* mutant abolished the ability to produce *N*-acyl homoserine lactones (AHL), quorum sensing molecules, whereas it activated the expression of *mexEFoprN*, genes for multidrug efflux pump (Taguchi et al. 2010). Other flagellum-related mutants, such as Δ*motABCD* and Δ*fgt1* also remarkably reduced the ability to produce AHL, and activated the expression of *mexEFoprN* (Kanda et al. 2011; Taguchi et al. 2010). We investigated the mechanisms and the effect of AHL production, and found possible relationships between quorum sensing, expression of *mexEFoprN* and flagellar motility. AHL production requires Gac two-component system and AefR transcription factor (Marutani et al. 2008; Kawakita et al. 2012). Both factors independently regulate AHL production. Because bacterial swarming motility requires flagella and type IV pili (T4P), we investigated phenotype of the Δ*pilA*, T4P-defective mutant (Taguchi et al. 2006; Taguchi and Ichinose 2011). The Δ*pilA* lost swarming motility, and reduced expression of *vfr*, a gene encoding virulence factor regulator, Vfr. Both Δ*pilA* and Δ*vfr* mutants retained the ability to produce AHL but reduced the expression of *hrp*-related genes and virulence toward tobacco plant (Taguchi and Ichinose 2013). Interestingly, Δ*psyR*, a mutant for autoinducer transcriptional regulator, activated the expression of *hrp*-related genes with unknown mechanisms (unpublished). We expect that the expression of each virulence-related transcription factor is complicatedly regulated during bacterial pathogenesis.

Key Words: AefR, Flagella, Multidrug efflux pump, Quorum sensing, Type IV pili.

REFERENCES

Felix G. et al. (1999) Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. Plant J. 18, 265–276.
Ichinose Y. et al. (2003) Need for flagella for complete virulence of *Pseudomonas syringae* pv. *tabaci*: genetic analysis with flagella-defective mutants Δ*fliC* and Δ*fliD* in host tobacco plants. J. Gen. Plant Pathol. 69 (4), 244-249.
Kanda E. et al. (2011) Two flagellar stators and their roles in motility and virulence in *Pseudomonas syringae* pv. *tabaci* 6605. Mol. Genet. Genomics 285 (2), 163-174.
Kawakita Y. et al. (2012) Characterization of each *aefR* and *mexT* mutant in *Pseudomonas syringae* pv. *tabaci* 6605. Mol. Genet. Genomics 287, 473-484.
Marutani M. et al. (2008) Gac two-component system in *Pseudomonas syringae* pv. *tabaci* is required for virulence but not for hypersensitive reaction. Mol. Genet. Genomics 279 (4), 313-322.
Taguchi F. et al. (2006) Identification of glycosylation genes and glycosylated amino acids of flagellin in *Pseudomonas syringae* pv. *tabaci*. Cell. Microbiol. 8 (6), 923-938.
Taguchi F. et al. (2010) Defects in flagellin glycosylation affect the virulence of *Pseudomonas syringae* pv. *tabaci* 6605. Microbiology 156 (1), 72-80.
Taguchi F. and Ichinose Y. (2011) Role of Type IV pili in virulence of *Pseudomonas syringae* pv. *tabaci* 6605: Correlation of motility, multidrug resistance, and HR-inducing activity on a nonhost plant. Mol. Plant-Microbe Interact. 24 (9), 1001-1011.
Taguchi F. and Ichinose Y. (2013) Virulence factor regulator (Vfr) controls virulence-associated phenotypes in *Pseudomonas syringae* pv. *tabaci* 6605 by a quorum sensing-independent mechanism. Mol. Plant Pathol. 14 (3), 279-292.

Flagella-independent motility of *Pseudomonas syringae* pv. *tomato* DC3000 and its contribution to virulence towards tomato plants

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Motility plays an essential role in bacterial fitness and colonisation in the plant environment. Benefits include increased efficiency of nutrient acquisition and avoidance of toxic substances, successful competition with other microorganisms, the ability to translocate to preferred hosts and access optimal sites within them, and the dispersal in the environment during the course of transmission. We have observed that *Pseudomonas syringae* pv. *tomato* DC3000 (*Pto* DC3000) moves over semisolid surfaces using at least two different types of motility: swarming, which depends on the presence of flagella and syringafactin, a biosurfactant produced by this strain, and a flagellum-independent surface spreading or sliding, which also requires syringafactin. This atypical mode of surface motility was revealed after deleting the flagellar master regulatory gene, *fleQ*, and was abolished in a *syfA* background, impaired in syringafactin production. We have investigated the role of motility in the virulence of Pto DC3000 towards tomato plants observing that mutants lacking flagella were as virulent as the wild type and only the simultaneous loss of both flagella and syringafactin impairs the ability of *Pto* DC3000 to colonize tomato host plants and cause disease. Also, we explore how syringafactin production is transcriptionally and post-transcriptionally regulated.

Key Words: Pseudomonas syringae, flagella, biosurfactant, swarming, sliding.

REFERENCES

BERTI, A.D., GREVE, N.J., CHRISTENSEN, Q.H., THOMAS, M.G. (2007): Identification of a biosynthetic gene cluster and the six associated lipopeptides involved in swarming motility of *Pseudomonas syringae* pv. *tomato* DC3000. Journal of Bacteriology 189, 6312-6323.
BURCH, A.Y., SHIMADA, B.K., BROWNE, P.J., LINDOW, S.E. (2010): Novel high-throughput detection method to assess bacterial surfactant production. Applied and Environmental Microbiology 76, 5363-5372.
BURCH, A.Y., SHIMADA, B.K., MULLIN, S.W., DUNLAP, C.A., BOWMAN, M.J., LINDOW, S.E. (2012): *Pseudomonas syringae* coordinates production of a motility-enabling surfactant with flagellar assembly. Journal of Bacteriology 194, 1287-1298.
BURCH, A.Y., ZEISLER, V., YOKOTA, K., SCHREIBER, L., LINDOW, S.E. (2014): The hygroscopic biosurfactant syringafactin produced by *Pseudomonas syringae* enhances fitness on leaf surfaces during fluctuating humidity. Environmental Microbiology 16, 2086-2098.
KEARNS, D.B. (2010): A field guide to bacterial swarming motility. Nature Reviews Microbiology 8, 634-644.
NOGALES, J., VARGAS, P., FARIAS, G.A., OLMEDILLA, A., SANJUÁN, J., GALLEGOS, M.T. (2015): Flagella-dependent and independent motilities of *Pseudomonas syringae* pv. *tomato* DC3000 and their contribution to virulence in tomato plants. Molecular Plant-Microbe Interactions Journal, submitted.
VARGAS, P., FARIAS, G.A., NOGALES, J., PRADA, H., CARVAJAL, V., BARÓN, M., RIVILLA, R., MARTÍN, M., OLMEDILLA, A., GALLEGOS, M.T. (2013): Plant flavonoids target *Pseudomonas syringae* pv. *tomato* DC3000 flagella and type III secretion system. Environmental Microbiology Reports 5, 841-850.

Involvement of type IV pili in *Acidovorax citrulli* virulence

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Acidovorax citrulli causes bacterial fruit blotch of cucurbits. Understanding the basic aspects of the interaction between *A. citrulli* and its hosts is demanded to develop efficient means to combat this threatening disease. *A. citrulli* produce type IV pili (TFP), thin, hair-like appendages found on the bacterial surface at the cell poles. TFP mediate a polar flagellum-independent type of bacterial surface motility termed twitching motility. Previous characterization of *A. citrulli* mutants impaired in *pilM* and *pilT* genes, revealed that type IV pili (TFP) are required for twitching motility and wild type levels of biofilm formation and virulence in this bacterium (Bahar et al., 2009). Further we generated and screened libraries of Tn*5* mutants seeking for alterations in colony morphology and twitching ability. About 20 new mutants affected in TFP genes and other genes that are indirectly connected to TFP biosynthesis and/or regulation were identified. Most characterized mutants showed reduced twitching motility, produced less biofilm and were less virulent than the wild type. Interestingly, a single mutant displayed larger twitching motility haloes than the wild type, though it was similarly affected in virulence and biofilm formation as the other mutants. In our lab we are also characterizing the phenomenon of phenotypic variation in *A. citrulli*, which occurs spontaneously during growth in culture as well as upon isolation from infected plants. Phenotypic variant (PV) colonies are bigger and fuzzier than the wild type ones, and in contrast to the latter, are not surrounded by twitching halos. In addition, PVs are compromised in their virulence. These traits resemble the phenotype of TFP mutants of this bacterium. Indeed, transmission electron microscopy revealed that the tested PVs lack the ability to produce TFP (Shrestha et al., 2013). Results from characterization of TFP mutants and PVs strengthen that TFP play a crucial rule in *A. citrulli* pathogenicity.

Key Words: Acidovorax citrulli, twitching motility, type IV pili, phenotypic variation.

REFERENCES

Bahar, O., Goffer, T., and Burdman, S. (2009). Type IV Pili Are Required for Virulence, Twitching Motility, and Biofilm Formation of *Acidovorax avenae* subsp. *citrulli*. Molecular Plant-Microbe Interactions 22, 909-920.
Shrestha, R.K., Rosenberg, T., Makarovsky, D., Eckshtain-Levi, N., Zelinger, E., Kopelowitz, J., Sikorski, J., and Burdman, S. (2013). Phenotypic Variation in the Plant Pathogenic Bacterium *Acidovorax citrulli*. PloS One 8, 16.

Perception of plant and environmental signals in *Pseudomonas syringae* pv. *tomato* DC3000

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Pseudomonas syringae pv *tomato* DC3000 (*Pto*) live outside the plant as saprophyte or epiphyte without causing disease until it is able to enter the plant through wounds or natural openings. Both outside and inside the plant, bacteria are subjected to a constant changing environment which exerts stressful conditions that threaten their survival. In order to prosper, *Pto* has to be able to sense environmental and plant signals to elaborate the optimal response to overcome these hostile conditions.

Light is revealing as a key regulator of lifestyle in non-phototrophic bacteria. *Pto* has a blue-light sensing LOV domain and two red-light sensing bacteriophytochromes. Previous work in our laboratory revealed that white light alters gene expression in *Pto* generating the up-regulation of adhesion genes and down-regulation of flagellar motility genes. To delve in the regulatory effect of light on *PsPto*, we have conducted a microarray hybridization experiment after the treatment with different monochromatic lights (blue and red). Preliminary results indicate that regulation of light perception can be controlled by the balance between blue and red light. Mutants in the LOV protein and in the bacteriophytochromes are being used to evaluate the role of the different photosensory proteins in the integrated network regulating light responses.

Entry process to the plant apoplast is crucial for *Pto* colonization of the host. Chemotaxis, which enables bacterial cells to move towards certain stimuli and away from others, has been described to drive the entry process in other phytopathogenic bacteria. We are carrying out a bioinformatics analysis followed by an experimental approach to identify putative *Pto* chemoreceptors involved in the perception of compounds released in plant wounds.

Key Words: *Pseudomonas syringae* pv *tomato* DC3000, light perception, chemoreceptors.

Functional characterization of the residues in regulatory proteins HrpG and HrpV of *Pseudomonas syringae* pv *tomato* DC3000

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P. syringae pv. *tomato* DC3000 utilises a Type III secretion system (T3SS) to transfer bacterial proteins into the plant host cell. T3SS assembly and activity is a highly controlled process regulated by proteins encoded by the *hrp*/*hrc* gene cluster, expression of *hrp*/*hrc* genes depends on HrpL extracytoplasmic sigma factor. The expression of hrpL gene is sigma 54 dependent and requires two transcriptional factors (enhancer binding proteins, EBPs) HrpR and HrpS (HrpRS). Activity of HrpRS is directly regulated by HrpV and HrpG proteins. HrpV is negative regulator of HrpRS while HrpG supresses HrpV negative regulation by binding HrpV. The HrpV changes the interactions between HrpR and HrpS required for transcriptional activation. Also HrpS associates simultaneously with HrpR and HrpV most likely playing a central role in the assembly of the repressed HrpRSV complex. HrpV can bind either HrpS or HrpG but not both simultaneously (Hutcheson et al., 2001; Wei CF et al., 2005; Jovanovic et al., 2011; Jovanovic et al. 2014). Recently it has been shown that HrpG and HrpV proteins can be co-purified and form a stable complex (Gazi A.D. et al. 2014). To establish structure-function relationships in HrpV and HrpG single alanine substitutions of conserved residues in HrpV and HrpG were constructed and HrpV and HrpG variants were analysed for the ability to interact with each other using the bacterial two hybrid system and for function using an in vivo *hrpL* promoter transcription assay. The results presented here reveal that HrpV by HrpG regulation appears dependent on residues found within their C terminus and that residues in HrpG L101 and L105 are indispensable for ability of HrpG to interact with HrpV and supress HrpV negative regulation.

Key words: HrpV, HrpG, HrpRS, σ54, DC3000

REFERENCES

HUTCHESON, SW.; BRETZ, J.; SUSSAN, T.; JIN, S.; PAK, K. (2001): Enhancer-binding proteins HrpR and HrpS interact to regulate *hrp*-encoded type III protein secretion in *Pseudomonas syringae* strains. J Bacteriol 183: 5589–5598.
WEI, CF.; DENG, WL.; HUANG, HC. (2005): A chaperone-like HrpG protein acts as a suppressor of HrpV in regulation of the *Pseudomonas syringae* pv. *syringae* type III secretion system. Mol Microbiol 57: 520–536.
JOVANOVIC, M.; JAMES, E.; BURROWS, P.; REGO, F.; BUCK, M.; SCHUMACHER. J. (2011): Regulation of the co-evolved HrpR and HrpS AAA+ proteins required for *Pseudomonas syringae* pathogenicity. Nature Communications doi: 10.1038/ncomms1177.
JOVANOVIC, M.; LAWTON, E.; SCHUMACHER, J.; BUCK, M. (2014): Interplay among *Pseudomonas syringae* HrpR, HrpS and HrpV proteins for regulation of the Type III secretion system. FEMS Microbiol Lett. 365(2): 201-211.
GAZI, A.; CHAROVA, S.; AIVALIOTIS, M.; PANOPOULOS, N.; KOKKINIDIS, M. (2014): HrpG and HrpV proteins from the Type III secretion system of *Erwinia amylovora* form a stable heterodimer. FEMS Microbiology Letters, 362, 1–8
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Pseudomonas syringae effectorless polymutants reveal novel interplay between effectors

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The bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 suppresses the two-tiered innate immune system of plants by injecting a complex repertoire of effector proteins into host cells via the type III secretion system. A functionally effectorless *Pst* DC3000 polymutant (DC3000D36E) lacking all 36 known effectors was created, sequence confirmed, and used to explore interplay among selected effectors. This strain retains a complete type III secretion system but differs from previously reported polymutant DC300028E in its interactions with *Nicotiana benthamiana*: DC3000D36E induces functional pattern-triggered immunity (PTI), elicits a flagellin-dependent 15-h burst of reactive oxygen species, and no longer elicits rapid cell death typical of effector-triggered immunity (ETI). These differences are entirely attributable to effector HopAD1, which is produced by DC3000D28E. Deleting hopAD1 from *Pst* DC3000 and heterologously expressing hopAD1 in *P. syringae* pv. *tabaci* 11528 demonstrated HopAD1 to act as an avirulence determinant in *N. benthamiana*. The ETI-like cell death triggered by HopAD1 in wild-type *N. benthamiana* and by AvrPto in *Pto* transgenic *N. benthamiana* was suppressed completely by AvrPtoBM3, an AvrPtoB variant that has site-specific mutations disrupting its E3 ubiquitin ligase domain and the two known domains for interacting with immunity-associated kinases. AvrPtoBM3 also gained the ability to interact strongly in a yeast two-hybrid system with immunity-kinase MKK2, whose production is needed for HopAD1- and AvrPto-dependent cell death. Natural variation in AvrPtoB-homolog (HopAB family) effectors revealed that loss of E3 ubiquitin ligase activity (indicated by ability to interact with the tomato Fen protein in yeast two-hybrid assays) correlated with strong interaction with MKK2 and strong suppression of HopAD1 ETI. Polymorphism in the E3 domain of HopAB family members suggests that these effectors have alternative, competing mechanisms for suppressing ETI, with an active E3 domain favoring Fen-dependent self-domain ETI suppression and an inactive E3 domain favoring other-effector ETI suppression.

Key Words: Pattern-triggered immunity, Effector-triggered immunity, Effector repertoire, Type III secretion system.

SESSION III

ECOLOGY

From crops to clouds and back again: the evolving story of a bacterium *sans frontiere*

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Since the first descriptions of *Pseudomonas syringae* as a plant-associated bacterium, the vision of its ecology has moved away from ubiquitous epiphytic plant pathogen to multi-faceted bacterium sans frontières in fresh water and other ecosystems linked to the water cycle. Discovery of the aquatic facet of its ecology has led to a vision of its life history that integrates spatial and temporal scales spanning billions of years and traversing catchment basins, continents and the planet, and that confronts the implication of roles that are potentially conflicting for agriculture - as a plant pathogen and as an actor in processes leading to rain and snowfall. This new ecological perspective has also yielded insight into epidemiological phenomena linked to disease emergence. It sets the stage for the integration of more comprehensive contexts of ecology and evolutionary history into comparative genomic analyses to elucidate how *P. syringae* subverts attack and defense responses of the cohabitants of the diverse environments it occupies. Here we will present our vision of the evolving story of the ecology and biology of *P. syringae* and we will speculate on how this story will continue to evolve in the future.

Beyond the symptom: the lifestyle of plant pathogenic pseudomonads when they are not inciting the disease.

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Plant pathogenic pseudomonads are diverse and versatile microorganisms. When they are not causing disease, strains of species such as *Pseudomonas syringae*, other than the water cycle, can survive and colonize the organ surfaces of host and non host plants, plant apoplast, pollen, seed as well as soil, rhizosphere and aphids. These habitats are quite various and pose different challenges for the rapid adaptation of the bacterial cell during the colonization of such environments. The survival on leaves requires mechanisms for facing a range of stresses such as ultraviolet radiation, desiccation, osmotic stress, water availability and temperature changes. Water availability on the leaf surface plays a fundamental role in determining the cell size for inducing the quorum sensing regulation system. The epiphytic fitness is enhanced by production of siderophores, alginate, levan and cellulose. Phytopathogenic pseudomonads also display considerable sets of antimicrobial compounds to counteract other environment microorganisms. Leaf surface colonization promotes flagellar and swarming motility and also chemosensing and chemotacting, whereas the apoplast fluid incites the synthesis of phytotoxins, syringolin A and degradation of γ-amino-butyric acid (GABA). Once inside the plant, pathogenic pseudomonads can reside for a period in the apoplast and/or tissues without causing any apparent symptom of disease. This latency phase can endure more than one year but change(s) in plant metabolism and/or in climatic predisposing factors can suddenly incite the appearance of disease symptoms. Whilst seed is a well known way of pathogen dispersal on long distances, pollen and aphids are still underestimate vectors for the phytopathogenic pseudomonads spreading and transmission. *P. syringae* would seem a poor soil survivor, even though some pathovars can effectively colonize host and non host rhizospheres and initiate the crop colonization through the root system. In contrast, *P. cichorii* and *P. viridiflava* are able to survive in soil and have metabolic pathways to degrade potent synthetic fumigant such as 1,3-dichloropropene, and the synthetic pyrethroid fenvalerate, respectively. *P. corrugata* appears as a very versatile microorganism being able to cause economically important diseases, produce biopolyesthers and reduce the chromium content in polluted soils. The globalized economy characterized by the continue movement and exchanging of plants or plant parts from different geographic areas can greatly contribute to the emergence of novel plant-pathogen interactions. The likelihood of an host shift depends on the global ecology governing the "triangle" of disease: pathogen, host and environment. A detectable and measurable distinct ecology of taxonomically closely-related strains could serve and help to demarcate plant pathogenic *Pseudomonas* species.

Are lichens natural reservoirs for plant pathogens?

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Although generally described as bipartite mutualistic associations of a fungus and an alga or cyanobacterium, lichens are also known to harbour species-specific communities of endo-lichenic bacteria, typically dominated by Proteobacteria. The functional roles and colonization mechanisms of these endolichenic bacteria are largely unknown. Among lichen-associated bacteria, revealed by both metagenomic and culture-based studies, are several known or suspected plant pathogens, including *Pseudomonas syringae*, *Erwinia persicina*, *Xanthomonas campestris*, and *Burkholderia glathei*, which invites speculations as to whether lichens serve as natural reservoirs for these pathogens in some environments. However, plant growth-promoting bacteria, such as *Pseudomonas fluorescens* and *Burkholderia phytofirmans*, are also commonly encountered in the lichen-associated microbiome, underscoring the complex impact that these oft-ignored members of the vegetation may have on plant health in natural environments.

Key Words: Lichen-associated bacteria, Endothallic bacteria, Symbiosis, Environmental reservoirs, Metagenomics.

REFERENCES

SIGURBJÖRNSDÓTTIR, M. A.; ANDRÉSSON, Ó. S.; VILHELMSSON, O. (2015): Analysis of the *Peltigera membranacea* metagenome indicates that lichen-associated bacteria are involved in phosphate solubilization. Published ahead of print in Microbiology, March 3, 2015. DOI: 10.1099/mic.0.000069.
GRUBE, M.; BERG, G.; ANDRÉSSON, Ó. S.; VILHELMSSON, O.; DYER, P. S.; MIAO, V. (2013): Lichen genomics: prospects and progress. In MARTIN, F. (ed.) Ecological Genomics of Fungi, 191-212. Wiley-Blackwell, Oxford, UK. ISBN: 978-1-119-94610-6.

A broad and operational classification framework to address the ecology of *Pseudomonas syringae* populations

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Understanding ecology of pathogens like *Pseudomonas syringae* is central to addressing the complexity of disease emergence and pathogen evolution and to develop effective and sustainable methods of control. An indispensable prerequisite is a classification tool that represents as much of the diversity as possible and delimits accurately the boundaries. By adopting an intensive sampling strategy from several continents and multiple substrates within and beyond agricultural zones we made reliable phylogroup delimitation through multi-locus sequence typing (MLST). We identified 23 clades of *P. syringae* within 13 phylogroups among which the seven previously described were included. The robustness of MLST-phylogroups was confirmed by the phylogeny of the core genome of representative strains. We demonstrated that the citrate synthase (*cts*) housekeeping gene can accurately predict phylogenetic affiliation for more than 97 % of more than 700 strains and we proposed a list of *cts* sequences to be used as a simple tool for quickly and precisely classifying new strains. We therefore proposed an expandable framework mainly based on a broad database and *cts* genetic analysis into which more diversity can be integrated. Phenotypic traits rarely provided means for classification even if some combinations were highly probable in some phylogroups. However, by clarifying the classification of strains from a wide range of habitats and describing the genotypic and phenotypic profiles of the different phylogroups, we revealed a fascinating diversity of life histories and adapative strategies deployed within the *P. syringae* complex. This has led us to also develop specific genetic markers, one allowing the assignment of strains to the *P. syringae* complex at large and others to the individual phylogroups. Using these markers in simple, rapid, reliable and cheap multiplex PCR will allow a medium throughput screen of *P. syringae* strains leading to easy assessment of population structure in a wide range of samples.

Key Words: Phylogeny, data base, classification tool, citrate synthase, specific PCR.

SESSION IV

EFFECTORS AND PLANT RESPONSES

Pseudomonas syringae type III effectors, their targets, and suppression of plant immunity

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The bacterial pathogen *Pseudomonas syringae* is dependent on a type III secretion system and the type III effector proteins (T3Es) it injects into plant cells to cause disease. The enzymatic activities of T3Es and their plant targets are not well understood. I will discuss the progress that we have made on T3E activities and plant targets. One T3E that will be discussed is HopE1, which strongly suppresses both effector-triggered and PAMP-triggered immunity. We found that HopE1 requires a eukaryotic co-factor that is needed for HopE1 to target a plant protein that functions in the microtubule network. Arabidopsis plants expressing HopE1 dissociate this protein from the microtubule network. Arabidopsis mutants lacking this target are more susceptible to *P. syringae* and exhibit reduced immune responses. Thus, HopE1 disables the microtubule network and one effect is that HopE1 inhibits protein secretion from plant cells, which is predicted to be beneficial to the pathogen because it inhibits the delivery of immunity-associated products to the apoplast. I will also discuss the progress that we have made on other *P. syringae* T3Es including an update on HopU1, which is a mono-ADP-ribosyltransferase that modifies several RNA-binding proteins including GRP7. We have found that over-expression of GRP7 protects plants to multiple types of pathogens. This benefit is greatly reduced in salicylic acid signaling and biosynthesis mutants. GRP7 binds to several different immunity-associated RNAs and enhances their translation. Thus, HopU1 benefits pathogenesis by inactivating an RNA-binding protein that helps translate immunity-associated RNAs into components of the plant immune response.

Key Words: effector biology, plant immunity.

Fate of *Pseudomonas syringae*-derived molecules in plants

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Pathogen-derived molecules, such as bacterial flagellin, are detected by plant receptors at the plasma membrane and induce local and systemic immune responses. Bacteria and other pathogens secrete effectors to counteract plant defenses and to promote the growth of the pathogen. Some of these effectors are recognized by plant intracellular receptors and induce stronger defenses, counteracted again by a new set of pathogen effectors. We study the fate of *Pseudomonas syringae*-derived molecules in Arabidopsis, their interactions with plant components, and plant responses to these molecules resulting in host immunity. PAMPs (pathogen associated molecular patterns) shed by pathogens in the apoplast may be endocytosed together with the plant plasma membrane receptors and accumulate inside the cells or move to distal areas. Bacterial effectors are injected directly to the host cells and can target multiple branches of defense responses by manipulation or use of plant components. Some effectors may modify other effectors to attenuate their defense inducing properties. Multiple players on both pathogen and host sides are contributing to the final outcome of defense susceptibility or local and systemic resistance.

Key Words: *P. syringae*, PAMPs, immunity, effectors

Outer membrane vesicles are potent inducers of plant immunity

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Gram-negative bacteria continuously release membrane vesicles, pinching off portions of their outer membrane. These outer membrane vesicles (OMVs) are an important component of the bacterial lifestyle and are involved in multiple processes including cell-cell communication, biofilm formation, stress tolerance, horizontal gene transfer and virulence. OMVs are also known modulators of the mammalian immune response. Despite the well-documented role of OMVs in mammalian-bacterial communication, their interaction with plants is not well studied. To examine whether OMVs of plant pathogens modulate the plant immune response we purified OMVs from three different plant pathogens (*Xanthomonas campestris*, *X. oryzae* and *Pseudomonas syringae*) and used them to treat *Arabidopsis thaliana*. OMVs rapidly induced ROS burst, defense gene induction and medium alkalinization in *A. thaliana* leaf discs, seedlings and cell cultures, respectively. Western blot analysis revealed that EF-Tu is secreted in OMVs and that it serves as an elicitor of plant immunity in this form. Our results further indicate that other immune elicitors both proteinaceous and non-proteinaceous, are present in OMVs and are responsible for the activation of the plant immune response. Taken together, our results reveal a new facet of plant-bacterial interactions and demonstrate that plants can detect and respond to OMV-associated elicitors by activation of their immune response.

Key Words: outer membrane vesicles, plant immunity, MAMPS, PAMPS, secretion.

REFERENCES

KULP, A.; KUEHN, M.J. (2010): Biological Functions and Biogenesis of Secreted Bacterial Outer Membrane Vesicles. Annual Reviews of Microbiology, 64, 163-184

Genetic and functional characterization of *Acidovorax citrulli* type III effectors

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Acidovorax citrulli (*Ac*) is a Gram-negative bacterium that cause bacterial fruit blotch (BFB) of cucurbits, a threatening disease of watermelon and melon industries worldwide. Despite the economic importance of BFB, still little is known about basic aspects of *A. citrulli*-host interactions (Burdman & Walcott, 2012). Based on biochemical and genetic features, most *Ac* strains can be divided into two major groups: group I includes strains that were mainly isolated from melon and other non-watermelon hosts, while group II includes strains that were mostly isolated from diseased watermelon (Walcott et al., 2000). As similar as several other Gram-negative pathogenic bacteria, *Ac* requires a functional type III secretion system (T3SS) for pathogenicity (Bahar & Burdman, 2010). The T3SS injects effector proteins into the plant cells, which collectively manipulate host cellular activities to the pathogen benefit. Due to the crucial role of type III secretion in *Ac* pathogenicity, we hypothesized that the distinguished host preferential association among group I and II strains, is associated with differences in the repertoire of type III-secreted effectors (T3Es). Indeed comparative analyses of T3E genes from strains isolated from various hosts and geographic locations revealed that overall, T3E genes cluster according to the group I/II classification. These analyses also revealed the existence of a third group of *A. citrulli* strains, which also differed from group I and II strains in other features (Eckshtain-Levi et al., 2014). We are currently using marker exchange mutagenesis combined with virulence assays, as well as heterologous expression in yeast to assess the role of individual effectors in virulence and host range determination in *Ac*. Bioinformatics approaches are also being employed to identify novel putative effectors of this pathogen.

Key Words: *Acidovorax citrulli*, bacterial fruit blotch, cucurbits, virulence, effectors.

REFERENCES

BURDMAN, S.; WALCOTT, R. (2012): *Acidovorax citrulli*: Generating Basic and Applied Knowledge to Tackle a Global Threat to the Cucurbit Industry. Molecular Plant Pathology, 13 (8), 805-815.

WALCOTT, R.R.; LANGSTON Jr., D.B.; SANDERS, Jr., F.H.; GITAITIS, R.D. (2000): Investigating Intraspecific Variation of *Acidovorax avenae* subsp. *citrulli* Using DNA Fingerprinting and Whole Cell Fatty Acid Analysis. Phytopathology, 90 (2), 191-196.

BAHAR, O.; BURDMAN, S. (2010): Bacterial Fruit Blotch: a Threat to the Cucurbit Industry. Israel Journal of Plant Sciences, 58 (1), 19-31.

ECKSHTAIN-LEVI, N.; MUNITZ, T.; ŽIVANOVIĆ, M.; TRAORE, S.M.; SPRÖER, C.; ZHAO, B.; WELBAUM, G.; WALCOTT, R.; SIKORSKI, J.; BURDMAN, S. (2014). Comparative Analysis of Type III Secreted Effector Genes Reflects Divergence of *Acidovorax citrulli* Strains Into Three Distinct Lineages. Phytopathology, 104 (11): 1152-1162.

Roles for the sensor-kinase RetS, small RNAs and divergent RsmA proteins in T3SS regulation

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The ECF sigma factor HrpL coordinates the concomitant expression of the T3SS complex and associated effector proteins necessary for suppression of defence responses [1]. In *Pseudomonas syringae*, the paralogous bacterial enhancer binding proteins HrpR and HrpS are both necessary for σ54-dependent transcription of *hrpL* [2] and HrpS is furthermore subject to feedback regulation by HrpV and HrpG [3]. However, how environmental and plant-derived signals are processed during HrpL expression remains unclear. The Gac/Rsm signalling network mediates lifestyle switching between T3SS-dependent and biofilm-associated infection states in *P. aeruginosa* [4] and evidence points to a similar role in *Ps* [5]. We report that the sensor histidine kinase RetS is required in order to suppress activity of the Gac-Rsm signalling network and thus enable *hrp*/*hrc* gene expression in DC3000. The ΔretS mutant is reduced in hrpL and hrpA promoter activities, synthesis of key effector proteins and virulence on the *A. thaliana* host. RNA-seq highlights significant overlap in the differential expression of T3SS genes in Δ*retS* and Δ*hrpL* mutants, further evidencing interlinked regulatory networks. Post-transcriptional regulation of target genes by the Gac/Rsm signal transduction pathway is mediated by the sequestration of the translational regulator protein RsmA (CsrA) by small regulatory RNAs [6]. Expression of all seven known GacA-dependent ncRNAs in DC3000 (*rsmX1-5*, *rsmY* and *rsmZ*) [7] is in-creased in the Δ*retS* transcriptome, suggesting that regulation of the T3SS regulon is like-wise associated with modulation of RsmA availability. The *P. syringae* genome contains four RsmA paralogues, two of which are expressed in T3SS-inducing conditions, suggesting that sub-functionalisation of both RsmA and sRNAs has enabled regulatory fine-tuning via combinatorial complexity. We show that the DC3000 RsmA paralogues differ in their ability to repress HrpL expression and utilise translational fusions and MRM-MS to suggest that this is mediated via post-transcriptional interactions with both HrpS and HrpV.

Key Words: T3SS, hrp, sRNA, Rsm, RNA-seq

REFERENCES

1. Hendrickson, E.L., P. Guevera, and F.M. Ausubel, The alternative sigma factor RpoN is required for *hrp* activity in *Pseudomonas syringae* pv. *maculicola* and acts at the level of *hrpL* transcription. Journal of Bacteriology, 2000. 182(12): p. 3508-3516.
2. Jovanovic, M., et al., Regulation of the co-evolved HrpR and HrpS AAA+ proteins required for *Pseudomonas syringae* pathogenicity. Nat Commun, 2011. 2: p. 177.
3. Jovanovic, M., et al., Interplay among *Pseudomonas syringae* HrpR, HrpS and HrpV proteins for regulation of the type III secretion system. FEMS Microbiology Letters, 2014. 356(2): p. 201-211.
4. Moscoso, J.A., et al., The *Pseudomonas aeruginosa* sensor RetS switches type III and type VI secretion via c-di-GMP signalling. Environmental Microbiology, 2011. 13(12): p. 3128-38.
5. Records, A.R. and D.C. Gross, Sensor Kinases RetS and LadS Regulate *Pseudomonas syringae* Type VI Secretion and Virulence Factors. Journal of Bacteriology, 2010. 192(14): p. 3584-3596.
6. Lapouge, K., et al., Gac/Rsm signal transduction pathway of gamma-proteobacteria: from RNA recognition to regulation of social behaviour. Molecular Microbiology, 2008. 67(2): p. 241-253.
7. Moll, S., et al., Construction of an *rsmX* co- variance model and identification of five *rsmX* non-coding RNAs in *Pseudomonas syringae* pv. *tomato* DC3000. RNA Biology, 2010. 7(5): p. 508-516.

Functions of the HopZ Type III Effector Family in *Arabidopsis*

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The YopJ / HopZ family of effector proteins is a common and widely distributed effector family found in both animal and plant pathogenic bacteria. The *P. syringae* HopZ family includes three major allele types (one ancestral and two brought in by horizontal gene transfer) whose diversification was driven by the host immune system. We have previously demonstrated that virulence and avirulence phenotypes in Arabidopsis are allele-specific and that the ZAR1 resistance protein recognizes HopZ1a in Arabidopsis. Our recent efforts to identify the host targets of the HopZ family and the ZAR1 resistance complex are shedding light on how this type III effector family has functionally diversified, and also identifying novel components of the plant immune system.

SESSION V

GENOMICS AND BIOINFORMATICS

Phylogenetic and VNTR analysis identified non-pathogenic lineages within *Xanthomonas arboricola* associated with Walnut lacking the canonical Type Three Secretion System.

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Pseudomonas syringae and *Xanthomonas arboricola* are conventionally known as taxons of epiphytic plant-pathogenic bacteria, including pathovars. We used *X. arboricola* as a model for investigating the population structure a plant associated bacterial species with a cultivated plant. This study showed that *X. arboricola* encompasses also non-pathogenic bacteria causing no apparent disease symptoms on their hosts. The aim of this study was to assess the *X. arboricola* population structure associated to walnut including non-pathogenic strains, in order to gain a better understanding of the role of non-pathogenic xanthomonads in walnut microbiota. A multi-locus sequence analysis (MLSA) was per-formed on a collection of 100 *X. arboricola* strains including 27 non-pathogenic strains isolated from walnut. Non-pathogenic strains grouped outside clusters defined by pathovars and formed separate genetic lineages. A multi-locus variable-number tandem repeat analysis (MLVA) conducted on a collection of *X. arboricola* strains isolated from walnut, showed that non-pathogenic strains clustered separately from clonal complexes containing *X. a.* pv. *juglandis* strains. The occurrence of the same genotype in different geographic areas supports the fact that non-pathogenic strains have been spread all over walnut growing areas in France. Some non-pathogenic strains of *X. arboricola* did not contain the canonical type III secretion system (T3SS) and harbored only one to three type III effectors (T3E) genes. In non-pathogenic strains CFBP 7640 and CFBP 7653, neither T3SS genes, nor any of the analyzed T3E genes were detected. This finding raises the question about the origin of non-pathogenic strains and evolution of plant pathogenicity in *X. arboricola*. T3E genes that were not detected in any non-pathogenic isolates studied represent excellent candidates to be those responsible for pathogenicity in *X. arboricola*.

Key Words: Walnut, microbial ecology, epiphytic bacteria, MLVA, MLSA.

Regulatory innovation rescues a catastrophic motility defect - an insight to bacterial robustness and resilience for a plant growth-promoting bacterium?

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Although *Pseudomonas fluorescens* hold great potential as biocontrol and plant growth promoting agents, their function and performance in the field are poorly understood. Motility is a key trait for bacterial lifestyle, to help them reach nutrients and evade predators. We observed that a *P. fluorescens* SBW25 *fleQ* (flagellum master regulator) and *viscBC* (viscosin synthase) mutant was unable to make a flagellum or produce viscosin and could not move on spreading motility medium (SMM). Viscosin was found to aid flagellum-dependent motility over plant roots and help protect seedlings germinating in the presence of oomycete root pathogens like *Pythium*. Although SBW25Δ*fleQ*::*visc* mutants, and equivalent Pf-01 strains, were unable to move from the point of inoculation on SMM, the starvation "catastrophe" facing the immotile bacteria selected for emergence motile mutants after 48-96h, seen as slow spreading colonies; fast-spreading blebs appeared from these colonies shortly afterwards. Genome re-sequencing revealed mutations in either *ntrB*, *glnA* or *glnK* in slow spreaders whereas the fast spreaders had the same initial mutation plus a secondary mutation in *ntrC*. We predict the first mutational changes raise intracellular levels of phosphorylated NtrC, which subsequently re-activates the flagellum genes and also switches on the nitrogen metabolism system. The constitutive activation of the nitrogen metabolism system results in compensatory mutations in the NtrC HTH DNA binding domain, which we postulate switches the protein specificity from *ntr* targets to flagellar gene targets. This alleviates the N-toxicity experienced by the cell, but the flagellar gene system is consequently upregulated and results in multiple flagella being synthesised from the cell pole. This work shows that *P. fluorescens* has the potential for use in biocontrol and highlights how bacteria can use rapid, repeatable innovations to re-wire its genetic circuitry to adapt to potentially catastrophic genome mutations – an important consideration for field robustness and resilience.

Key Words: Regulation, PGPR, Swarming motility, Sliding motility, Plant protection.

REFERENCES

ALSOHIM, A.S.; TAYLOR, T.B.; BARRETT, G.A.; GALLIE, J.; ZHANG, X.X.; ALTAMIRANO-JUNQUEIRA, A.E.; JOHNSON, L.J.; RAINEY, P.B.; JACKSON, R.W. (2014): The biosurfactant viscosin produced by *Pseudomonas fluorescens* SBW25 aids spreading motility and plant growth promotion. Environmental Microbiology, 16 (7), 2267-2281.
Taylor, T.B.; Mulley, G.; Dills, A.H.; Alsohim, A.S.; McGuffin, L.J.; Studholme, D.J.; Silby, M.W.; Brockhurst, M.A.; Johnson, L.J.; Jackson, R.W. (2015): Evolutionary resurrection of flagellar motility via rewiring of the nitrogen regulation system. Science, 347 (6225),1014-1017

Does bigger mean better? first lessons from comparative genomics in *Acidovorax citrulli*

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Comparative genomics between closely related species can assist us to discover genes that contribute to specific niche adaptation of certain species, and provide a better understanding of its evolution and distribution. *Acidovorax citrulli* (*Ac*), formerly *Pseudomonas pseudoalcaligenes* subspecies *citrulli*, is the causal agent of bacterial fruit blotch (BFB) of cucurbits. Serious economic losses due to BFB occurred in watermelon and melon in many parts of the world, and the disease represents a serious threat to the cucurbit industry [Burdman & Walcott, 2012]. Ac requires a functional type III secretion system (T3SS) for pathogenicity [Bahar & Burdman, 2010]. The T3SS injects effector proteins into the host cells, which collectively manipulate host cellular activities to promote disease. Comparative analyses of effector sequences from various *Ac* strains combined with additional experimental data support the existence of at least three groups within *Ac* [Eckshtain-Levi et al., 2014]. Currently we are performing comparative genome analysis between two model strains that represent the two major groups of *A. citrulli*, M6 (group I) and AAC00-1 (group II). Preliminary results revealed that 8 major genomic segments that are present in the AAC00-1 genome, ranging from ~30 to 120 kb, are absent in M6. Those segments lacking in the M6 genome explain why its size is ~500-Kb shorter than that of AAC00-1. Interestingly, strain M6 has been shown to be a highly aggressive strain in all cucurbits tested so far, including watermelon, whereas group II strains are strongly associated with watermelon and have moderate virulence on other cucurbits. We are now exploring whether these findings reflect broad differences among groups I and II strains. In parallel, we are employing machine learning to uncover new putative effectors that may provide further insights into host preferential differences between the two groups.

Key Words: *Acidovorax citrulli*, bacterial fruit blotch, comparative genomics, specific segments, machine learning.

REFERENCES

BURDMAN, S.; WALCOTT, R. (2012): *Acidovorax citrulli*: Generating Basic and Applied Knowledge to Tackle a Global Threat to the Cucurbit Industry. Molecular Plant Pathology, 13 (8), 805-815.
BAHAR, O.; BURDMAN, S. (2010): Bacterial Fruit Blotch: a Threat to the Cucurbit Industry. Israel Journal of Plant Sciences, 58 (1), 19-31.
ECKSHTAIN-LEVI, N.; MUNITZ, T.; ŽIVANOVIĆ, M.; TRAORE, S.M.; SPRÖER, C.; ZHAO, B.; WELBAUM, G.; WALCOTT, R.; SIKORSKI, J.; BURDMAN, S. (2014). Comparative Analysis of Type III Secreted Effector Genes Reflects Divergence of *Acidovorax citrulli* Strains into Three Distinct Lineages. Phytopathology, 104 (11): 1152-1162.

Transcriptome of the newly-sequenced *Ralstonia solanacearum* strain UY031 infecting the wild potato *Solanum commersonii*

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Ralstonia solanacearum is the causative agent of the bacterial wilt disease on a large number of plant species and is considered as one of the most devastating bacterial plant pathologies worldwide. Due to its agronomical impact, it is important to study specific plant-pathogen interactions in order to better understand how the pathogen modulates the outcome of the infection at a genetic level. In this work, we studied the interaction of *R. solanacearum* UY031, a very aggressive strain isolated from potato, with the wild potato *Solanum commersonii*. To this end, we sequenced the genome of strain UY031 and present here the first fully closed genome of a phylotype IIB1 strain and the fourth of *R. solanacearum*. We describe the Type III Effector (T3E) repertoire of UY031 and compare it to other completely and partially sequenced strains. Furthermore, we have used RNAseq to study the transcriptome of bacteria grown in rich medium and that of bacteria growing inside plant tissues. In the latter case, bacterial transcripts were obtained after deep sequencing of total RNAs isolated from infected and non-infected potato tissues and in silico read selection. With this information, we provide new insights in the understanding of *R. solanacearum* mode of infection, and define genes that are necessary and specific for a successful interaction.

Key Words: *Ralstonia solanacearum*, Bacterial wilt, Type III Effector, complete genome sequence, RNA sequencing.

Complete sequence and comparative genomic analysis of eight native *Pseudomonas syringae* plasmids belonging to the pPT23A family

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Pseudomonas syringae is a common foliar bacterium and causal agent of plant diseases in many different hosts worldwide. This species comprises over 60 pathovars and their host range differentiation has been analysed routinely based on the repertoire of specific type III effectors (Lindeberg et al 2009; Baltrus et al 2012). Genomic analyses have revealed that some genetic traits have been acquired through horizontal gene transfer and that the location of many of these genes are adjacent to mobile genetic elements. Plasmids are considered as major mobile genetic elements that contribute to genome innovation and evolution (Norman et al 2009). The pPT23A plasmid family (PFPs) comprises plasmids that appear to be indigenous to *P. syringae* and related bacteria and contribute to the ecological and pathogenic fitness of their *P. syringae* hosts (Sundin 2007). In this study we generated closed complete sequences of eight pPT23A-family plasmids, six of them isolated from *P. syringae* pv. *syringae* strains from different hosts, one from *P. syringae* pv. *garcae* and one from *P. syringae* pv. *tabaci*. We performed a comprehensive bioinformatic analysis of these plasmids in order to further understanding the evolution of the pPT23A plasmid family and the role of these plasmids in *P. syringae* biology and pathogenesis. Phylogenetic analysis revealed the presence of a new group of plasmids, and synteny analysis showed that *repA*, *rulAB* (except plasmid pPg2708), type IV secretion systems and genes related with plasmid stability are present in the same location in the different plasmids. Specific and essential genetic determinants have been also found (e.g. multiple type III effectors, different structures of copper resistance operon, *luxR* transcriptional regulators, etc). To date, the availability of closed plasmid sequences from *P. syringae* is limited, so, this study contributes to unravel the genetic bases of the role of PFPs in different *P. syringae* lifestyles.

Key Words: *P. syringae*, pPT23A plasmid family, type III effectors, *rulAB*, *copABCD*

REFERENCES

Baltrus, D.A; Nishimura, M.T.; Dougherty, K.M.; Biswas, S.; Mukhtar, M.S.; Vicente, J.; Holub, E.B.; Dangl, J.L. (2012): The molecular basis of host specialization in bean pathovars of *Pseudomonas syringae*. Molecular Plant-Microbe Interactions, 25 (7), 877-888.
Lindeberg, M.; Cunnac, S.; Collmer, A. (2009): The evolution of *Pseudomonas syringae* host specificity and type III effector repertoires. Molecular Plant Pathology, 10 (6), 767-775.
Norman, A.; Hansen, L.H.; Sørensen, S.J. (2009): Conjugative plasmids: vessels of the communal gene pool. Philosophical Transactions B, 364, 2275-2289.
Sundin G.W. (2007). Genomic insights into the contribution of phytopathogenic bacterial plasmids to the evolutionary history of their hosts. Annual Review of Phytopathology, 45, 129-151

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SESSION VI

EMERGING RESEARCH AREAS

The apoplastic arena in defence and disease

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The apoplastic compartment is the arena in which endophytic pathogens such as *P. syringae* grow and interact with plant cells. We have developed a robust protocol for the extraction of apoplastic washing fluid (AWF) from plant leaves (O'Leary et al 2014), which we have used in conjunction with a combination of metabolomic, proteomic and ion analysis techniques to describe the composition of the apoplast of *Phaseolus vulgaris* leaves, and to determine the changes in apoplast composition associated with resistant and susceptible interactions with the bacterial pathogen *Pseudomonas syringae* pv. *phaseolicola* (*Pph*). We have also used metabolic footprinting to identify metabolites that are depleted or excreted by *Pph* during growth in AWF. Overall, infection with a virulent strain of *Pph* was found to result in relatively minor changes in apoplast composition, allowing *Pph* to thrive in an environment to which it is well-adapted. However, elicitation of effector-triggered immunity (ETI) in resistant plants was found to result in wide-ranging and rapid changes in apoplastic ions and metabolites that, while potentially increasing nutrient abundances available to *Pph*, may also be associated with plant defence responses. Comparison of these results with data obtained using the tomato pathogen *P. syringae* pv. *tomato* highlights intriguing differences in the behaviour of these two pathogens that may reflect adaptation within *P. syringae* lineages to specific host environments.

Key Words: *Pseudomonas syringae* pv. *tomato*, *Pseudomonas syringae* pv. *phaseolicola*, metabolomics, proteomics, metabolic flux analysis.

REFERENCES

O'Leary, B.M., Rico, A., McCraw, S., Fones, H.N., and Preston, G.M. (2014) The infiltration-centrifugation technique for extraction of apoplastic fluid from plant leaves using *Phaseolus vulgaris* as an example. Journal of Visualised Experiments e52113.

Molecular analysis of the role of LscR, a small regulatory protein involved in expression of levansucrase in *Pseudomonas syringae* pv. *glycinea* PG4180

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Pseudomonas syringae is a phytopathogenic γ-proteobacterium that induces a wide variety of leaf spots, speck, and blight diseases on various agronomically significant crops, as well as on an unknown number of wild plant species (Srivastava, Al-Karablieh et al. 2012). Virulence of the bacterial blight pathogen of soybean, *Pseudomonas syringae* pv. *glycinea* PG4180, is favored by the temperature dependent production of levansucrase, an enzyme needed for production of the exopolymer levan and encoded by *lscB* (Li, Schenk et al. 2006, Mehmood, Abdallah et al. 2015). Expression of *lscB* in the heterologous host, *Pseudomonas putida* KT2440, required a PG4180-borne gene designated *lscR* (Zhurina 2009). Our preliminary research focused on understanding the role of *lscR* in expression of levansucrase. Different plasmid constructs were transformed into *P. putida* KT2440 wild type and then grown on MG plates containing 5% Sucrose. Plates were incubated over-night at 28°C then transferred to 18°C and left for 10 days. Our results showed that transformants carrying either *lscR* or *lscB* are levan negative. However, when *P. putida* KT2440 carried both *lscR* and *lscB*, a positive levan phenotype was observed characterized by a white dome shaped colony. An interesting additional phenotype was observed when we compared the effects of two plasmid constructs: one carrying only a 1,350-bps region containing *lscR*, the other harboring a ~25-kb region contained in a cosmid and flanking *lscR*. The *P. putida* transformant carrying the 1,350-bps lscR region showed a 'swimming-out' phenotype. In contrast, the P. putida transformant carrying the cosmid showed a regular, evenly edged colony morphology without signs of swimming. Because of the exciting phenotypes, current focus is on creating a PG4180 *lscR* mutant to investigate its effect on levansucrase expression. Moreover, in planta assays of PG4180 and its *lscR* mutant will be performed on soybeans plants to investigate whether *lscR* gene plays a role in the virulence of PG4180.

Key Words: Soybean, *Pseudomonas syringae*, Levansucrase, LscR

REFERENCES

Li, H., A. Schenk, A. Srivastava, D. Zhurina and M. S. Ullrich (2006). "Thermo-responsive expression and differential secretion of the extracellular enzyme levansucrase in the plant pathogenic bacterium *Pseudomonas syringae* pv. *glycinea*." FEMS Microbiol Lett 265(2): 178-185.

Mehmood, A., K. Abdallah, S. Khandekar, D. Zhurina, A. Srivastava, N. Al-Karablieh, G. Alfaro-Espinoza, D. Pletzer and M. S. Ullrich (2015). "Expression of extra-cellular levansucrase in *Pseudomonas syringae* is controlled by the in planta fitness-promoting metabolic repressor HexR." BMC Microbiol 15(1): 349.

Srivastava, A., N. Al-Karablieh, S. Khandekar, A. Sharmin, H. Weingart and M. S. Ullrich (2012). "Genomic Distribution and Divergence of Levansucrase-Coding Genes in *Pseudomonas syringae*." Genes 3(1): 115-137.

Zhurina, D. (2009). Identification and partial characterization of transcriptional regulators involved in temperature-dependent expression of levansucrase in *Pseudomonas syringae*. PhD Thesis, Jacobs University Bremen.

A VAPBC toxin-antitoxin module is involved in stress response of the plant pathogen *Acidovorax citrulli*

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Toxin-antitoxin systems are commonly found on plasmids and chromosomes of bacteria and archea. These systems appear as biscystronic genes encoding a stable toxin and a labile antitoxin, which protects the cells from the toxin's activity. Under specific conditions, such as stress or initial host infection the unstable antitoxin is degraded - the toxin becomes active and growth is arrested.
Using genome analysis we identified a putative toxin-antitoxin encoding system in the genome of the plant pathogen *Acidovorax citrulli.* Phylogenetic analyses suggested that this loci is unique to group II *A. citrulli* and several Xantomonads, and that it is not found in other *Acidovorax* sequenced species. Using biochemical and molecular analyses we show that *A. citrulli* VapBC module is a bona-fide toxin-antitoxin module in which VapC is a toxin with ribonuclease activity that can be counteracted by its cognate VapB protein. We further show that transcription of the *vapBC* loci is induced by amino acid starvation, chloramphenicol, and during plant infection.
Due to the possible role of TA systems in both virulence and dormancy of human pathogenic bacteria, studies of these systems are gaining a lot of attention. Conversely, studies of toxin-antitoxin systems in plant pathogenic bacteria are lacking. The study presented here validates a role for *vapBC* proteins in *A. citrulli* growth regulation, and suggests an involvement in host-pathogen interactions. To the best of our knowledge this study is the first systematic analysis of a chromosomal encoded *vapBC* of a plant pathogenic bacterium.

Key Words: *Acidovorax citrulli*, Toxin Anti-toxin, VapBC, Plant pathogen.

Ca^{2+} transport in *Pseudomonas savastanoi* pv: *savastanoi*, the causal agent of olive knot disease

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Pseudomonas savastanoi pv. *savastanoi* (*Psv*) has the capacity to leave as epiphyte on phyllosphere and to penetrate host tissues through wounds. Once inside plant tissue, *Psv* colonize the apoplast and, due to his ability to produce auxins and cytokinins, give raise to knots formation. Little is known about the molecular signals that are perceived in the apoplast by the plant pathogenic bacteria, which probably are involved in the adaptation to this environment very poor in nutrients. Through the use of fluorescent probes FURA 2AM we observed that *Psv* (strain DAPP-PG 722) communicates in vitro with the extracellular medium through the variations of the cytosolic Ca^{2+} levels, an important secondary messenger involved in various cellular activities and in plant-pathogen interaction. Preliminary results indicate a relationship between a possible state of energy deficiency in the cells and Ca^{2+} signals. In fact, when the bacterial cells were incubated in the absence of glucose, fructose, and sorbitol, which are present at very low concentrations in the apoplast, we observed an increase in the cytosolic Ca^{2+} due to the entry of this ion from the extracellular medium. We have also demonstrated that the channel for the entry of extracellular Ca^{2+} in the bacterium is: i) a Na^{+}/Ca^{2+} exchanger, since it is inhibited by Li^{+} ions employed in place of Na^{+}; ii) antagonized by nifedipine, inhibitor of the L-voltage channels responsible for the entry of the extracellular Ca^{2+} in mammals. In silico analysis of the genome of *Psv* strain DAPP-PG 722 revealed the presence of a gene coding for a Na^{+}/Ca^{2+} exchanger, belonging to the Na^{+}/Ca^{2+} exchanger family protein integrated membrane. Studies are in progress to obtain and characterise *Psv* mutants unable to produce such Na^{+}/Ca^{2+} exchanger, to verify if the mutation affects the virulence of the bacterium.

Key Words: olive knot, *Pseudomonas savastanoi* pv. *savastanoi*, cytosolic calcium, virulence.

Manipulation of Apoplastic Hydrolases by *Pseudomonas syringae*

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Plants respond to pathogen infection by secreting a battery of hydrolases that include proteases, lipases and glycosidases. These enzymes may contribute to the immune response by hydrolysing pathogen structures, and/or by releasing pathogen- or host-derived elicitors that amply the immune response. We hypothesise that, when living in the apoplast during infection, *P. syringae* suppresses the activity of these host hydrolases by secreting inhibitors. We develop and apply activity-based protein profiling to demonstrate the suppression of various apoplastic hydrolases during infection and are currently characterizing inhibitors of C14/Pip1 proteases; subtilases and beta-galactosidases. Depletion of the host hydrolase often increases bacterial growth, whilst inhibitor depletion reduces virulence, demonstrating that apoplast manipulation is important for virulence by *P. syringae*.

SESSION VII

EVOLUTION, TAXONOMY AND SYSTEMATICS

A persistent reservoir of a genomic island in *Pseudomonas syringae* pv. *phaseolicola*

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The bean halo blight pathogen *Pseudomonas syringae* pv. *phaseolicola* (*Pph*) strain 1302A carries a chromosomal 106kb Integrative and Conjugative Element (ICEland - a type of genomic island), designated PPHGI-1, encoding effector gene *avrPphB*. Bean cultivar Tendergreen has evolved to recognise *avrPphB* upon 1302A infection resulting in a rapid hypersenstive reaction (HR), which imposes strong selection on 1302A to evolve inactivation of *avrPphB* through island excision and loss. The emergent evolved strain, RJ3, increases in frequency within the 1302A population, such that the population can cause water-soaking disease lesions (Pitman et al., 2005). However, 0.5% of the population is 1302A containing PPHGI-1. We developed a mathematical model, which predicted PPHGI-1 would be maintained in the population long term if it conferred a fitness advantage. We empirically tested the predictions made by the model and determined that PPHGI-1 frequency in the bacterial population drops until the HR is not triggered and PPHGI-1 is stably maintained in 0.5% of the population over the long term. However, when a 99.5:0.5% RJ3:1302A population is inoculated into a susceptible bean cultivar that does not produce the HR, the frequency of PPGHI-1-carrying cells increases rapidly suggesting that PPHGI-1 confers a fitness benefit. We also observed that PPHGI-1 can be maintained in the bacterial population in a circular episomal form (Godfrey et al., 2011). When this happens, the PPHGI-1 episome replicates autonomously and *avrPphB* exhibits reduced expression levels, to help it evade host resistance (Lovell et al., 2010). We postulate that PPHGI-1 has evolved a hybrid transposon-plasmid structure that ensures its long term survival, by recombination into chromosomes and provision of enhanced bacterial fitness, or by using its plasmid properties to mask its genes and persist at low frequency over the long term. These results provide insights into the long term population dynamics of ICEland retention and loss.

Key Words: Genomic island, virulence, evolution, mobile genetic element.

REFERENCES

GODFREY, S.A.C.; LOVELL, H.C.; MANSFIELD, J.W.; CORRY, D.S.; JACKSON, R.W.; AR-NOLD, D.L. (2011): The stealth episome: suppression of gene expression on the excised genomic island PPHGI-1 from *Pseudomonas syringae* pv. *phaseolicola*. PLoS Pathogens, 7(3): e1002010.
LOVELL, H.C.; JACKSON, R.W.; MANSFIELD, J.W.; GODFREY, S.A.C.; HANCOCK, J.T.; DESIKAN, R.; ARNOLD, D.L. (2010): In planta conditions induce genomic changes in *Pseudomonas syringae* pv. *phaseolicola*. Molecular Plant Pathology 12,167-76.
PITMAN, A.; JACKSON, R.W.; MANSFIELD, J.M.; KAITELL, V.; THWAITES, R.; ARNOLD, D.L.; (2005) Exposure to host resistance mechanisms drives evolution of bacterial virulence in plants. Current biology. 15, 2230-2235.

Comparative & evolutionary genomics of the *Pseudomonas syringae* species complex

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Pseudomonas syringae encompasses a common and highly diverse species complex that includes members that can infect numerous agricultural and wild plant species. The recent wide-spread application of next-generation sequencing technology has driven a remarkable expansion in our understanding of the genetic structure of this species complex, yet we still have only a limited understanding of the specific genetic factors underlying niche specificity. Here we present our comparative and evolutionary analysis over >400 strains of *P. syringae*, including the pathotype strain collection. We specifically focus on the compositional dynamics of the genome, the impact of selection and recombination on genes associated with host adaption, and the use of association tests to identify previously unrecognized genes with host-specific associations. We find that virulence-associated genes are more likely to be recombinogenic and under selection, as well as a strong signal for inter-pathogroup recombination. We argue that this inter-pathogroup recombination of niche-associated genes is critical for maintain genetic cohesion and thereby delimiting the species complex.

Key Words: Comparative Genomics, Evolutionary Genomics, Natural Selection, Recombi-nation, Association Genetics.

Exploring the genetic basis of host specificity in *Pseudomonas syringae*

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The globally important phytopathogen, *Pseudomonas syringae*, includes pathovars that infect over 180 plant species. Individual pathovars only infect one or a few hosts. However, despite this specialisation, host jumps have occurred frequently within *P. syringae*. It is believed that effector repertoires are linked to host range and therefore genetic alteration of these repertoires may enable host range expansion. This topic was explored using three divergent clades that have convergently evolved to cause bacterial canker on *Prunus* species: *P. syringae* pv. *morsprunorum* (*Psm*) (which is differentiated into two races) and *P. syringae* pv. *syringae* (*Pss*). A *P. syringae* strain isolated from *Aquilegia vulgaris* is closely related to *Psm* R2, suggesting a host jump event. The genomes of *Psm* R1, R2, *Pss* and the *Aquilegia* strain were sequenced using Illumina Mi-Seq. Comparative genomics of the *Prunus* strains has revealed highly divergent effector repertoires within and between the different clades infecting *Prunus* species. They also differed in the presence of phytotoxin genes and those associated with survival in woody plant tissue. This analysis indicates that they may utilise different virulence mechanisms to cause similar disease outcomes. Further bioinformatics and cloning approaches will be used to find genes contributing to host specificity and pathogenicity.

Key Words: Host-specificity, effector, resistance, *Prunus*, genomics

An integrated approach of diagnostic to detect inter and intraspecific diversity in *Erwinia*, *Xanthomonas* and *Pseudomonas*

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Integrated diagnostic of plant bacterial diseases combines several specific screening tools such as real-time PCR, LAMP or serological techniques with isolation, followed by phenotypic and genotypic identification of phytopathogenic bacteria, enhancing the accuracy of their diagnostic. The use of these integrated approaches has allowed to discover key differences among strains similar to well known species but showing distinct characteristics in molecular and/or serological and/or or identification tests, including pathogenicity. The discovery of two new species as well as new variants of some bacterial species isolated from diseased plants is presented. The first example is *Erwinia piriflorinigrans*, a new pathogenic species described in Spain as causal agent of necrosis of pear blossoms and recently isolated from apple and other rosaceous hosts, that is closely related to *E. amylovora*, sharing in some cases the same habitat. The second example is a new *Xanthomonas* sp., also isolated in Spain but from peach and responsible of symptoms similar to those caused by *Xanthomonas arboricol*a pv. *pruni*, although classified as different to such species according to multilocus sequence analysis, serology and PCR. The third example are the different types of *Pseudomonas syringae* isolated from kiwi in North Spain that show low virulence and different molecular characteristics than those of the virulent type of *P. syringae* pv. *actinidiae*. The description of these new species or infraspecific groups leads to a more comprehensive knowledge of the complexity of pathosystems in cultivated crops, demonstrating that similar symptoms can be caused by more than one pathogen and that new variants can also be observed. Moreover, the partial or complete genomes of these new taxons that are being determined will give clues about the evolution of bacterial species, offer new omics-based tools for improving detection, identification or taxonomy and will provide new data on the life cycle and ecology of bacterial plant pathogens.

Key Words: *Erwinia piriflorinigrans*, *Pseudomonas syringae* , *Rosaceae*, stone fruits, kiwi

REFERENCES

López M. M. et al. 2011. *Erwinia piriflorinigrans* sp. nov., a novel pathogen that causes necrosis of pear blossoms. Int. J. System. Evol. Microbiol. 61:561-567.

Relevant features to epiphytic and pathogenic lifestyles from the genome sequence of *Pseudomonas syringae* UMAF0158.

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Pseudomonas syringae is one of the more extended and better adapted plant pathogen known until day. The introduction of mango crops in south of Spain has involved the presence of pathovar *syringae* strains adapted to this tropical plant, that in favourable environmental conditions can produce the apical necrosis disease. The complete sequence and annotation of the *P. syringae* pv. *syringae* (*Psy*) UMAF0158 chromosome and its native 62-kb plasmid pPSS158 has been obtained. Comparative bioinformatics analysis with the genomes of other sequenced *P. syringae* strains was performed in order to identify relevant aspects of this strain. We identified several genetic features, which are absent in the genome of *Psy* B728a, that may explain the ability of *Psy* UMAF0158 to interact with mango trees; such as, the mangotoxin biosynthetic operon *mbo*, a gene cluster for cellulose production, two different type III and two type VI secretion systems, and a particular T3SS effector repertoire. A mutant strain defective in the atypical T3SS showed no differences compared to wild-type strain during its interaction with host and non-host plants and worms. On the other hand, the functionality of the cellulose biosynthetic operon has been confirmed and its contribution to the formation of biofilm and adhesion to the mango leaf surface proved, suggesting that this operon favours epiphytic fitness. The relevance of mangotoxin production associated with the *mbo* operon in virulence has been also confirmed.

Key Words: Genome sequence, cellulose, mangotoxin, type III secretion system, colonization.

COST SUSTAIN SESSION ON EFFECTORS AND PLANT RESPONSES

Bacterial type-III effectors and the suppression of plant immune signalling.

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Most bacterial plant pathogens employ a type-III secretion system to inject type-III effector (T3E) proteins directly inside plant cells. These T3Es manipulate host cellular processes in order to suppress immune responses and create a permissive niche for bacterial proliferation, allowing development of the disease. In previous work, we found that *Pseudomonas syringae* employs the tyrosine phosphatase HopAO1 to inhibit tyrosine phosphorylation of the plasma membrane-localised immune receptors EFR and FLS2, thus suppressing the activation of innate immune responses. Interestingly, the virulence contribution of HopAO1 is considerably reduced, but not completely abolished, in the absence of EFR and FLS2, indicating that targeting these receptors is an important component of HopAO1 virulence activities, but also that other targets may play a relevant role in the contribution of HopAO1 to the infection. This supports the notion that specific T3Es may play numerous independent roles inside plant cells, and, in fact, different T3E activities may be specifically relevant at different stages of the infection process, globally contributing to the establishment of disease. The development of holistic approaches to study effector biology during the infection process constitutes an exciting challenge for future studies.

Key Words: Effectors, Immunity, Phosphorylation, Plant Signaling.

Cellular dynamics of HopQ1, a type III secretion effector from *Pseudomonas syringae*

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HopQ1 (for Hrp outer protein Q), a TTSS effector secreted by *Pseudomonas syringae* pv. *phaseolicola*, promotes the development of halo blight in bean. Upon delivery into a plant cell, HopQ1 undergoes phosphorylation and binds host 14-3-3 proteins. This interaction affects subcellular localization and stability of the effector contributing to virulence of bacteria.
Mass spectrometry analysis of an ectopic variant of HopQ1 demonstrated that the effector is phosphorylated in host cytoplasm. Size exclusion chromatography coupled to MALS (Multi-Angle Light Scattering) revealed that HopQ1 forms *in vitro* monomers, dimers and trimers. Treatment with a reducing agent or mutations in either one of both HopQ1 cysteines abolished oligomerization, indicating that HopQ1 oligomeric state depends on disulfide bridge formations. Following calcium depletion HopQ1 monomers were reversibly converted to dimers. Consistently, mutations in the predicted calcium binding motif of HopQ1 (HopQ1-D107A_D108A) produced HopQ1 dimers *in vitro* and *in planta*. We observed an increase in the nuclear rate of the HopQ1-D107A_D108A compared to wild-type HopQ1, which localizes primarily to the cytoplasm. This may indicate that dimer assembly promotes nuclear localization of HopQ1. Consistent with this model, co-expression of HopQ1-RFP (red fluorescent protein) fusion together with HopQ1-YFP (yellow fluorescent protein) fused to a nuclear localization signal (NLS) led to the nuclear accumulation of both proteins. These results strongly suggest that HopQ1 is translocated into the nucleus as a dimer.
Therefore, we propose a model in which cellular dynamics of HopQ1 is determined by phosphorylation-dependent interaction with 14-3-3s and calcium and/or redox-dependent oligomerization of the effector.

Key Words: TTSS effector, 14-3-3, phosphorylation, oligomerization, calcium.

Investigating the role of chromatin remodeling in plant immunity

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Plants are sessile organisms exposed to a variety of environmental challenges including pathogen infection. During pathogen attack, plants rapidly respond to infection via the recruitment and activation of immune complexes. Despite our extensive understanding of how plants perceive pathogens, little is known about the downstream responses leading to immunity. Part of the immune responses to pathogens is reprogramming of gene expression. A major mechanism controlling the modulation of gene expression is chromatin remodeling. Our data demonstrate that chromatin remodeling is part of the early responses following extracellular pathogen perception. Chromatin remodeling requires post-translational histone modifications such as acetylation, methylation, ubiquitination or phosphorylation. We are currently investigating the role of histone acetyltransferases (HATs) in pathogen-associated molecular patterns (PAMPs)-triggered immunity. Finally, our data indicate that nuclear localized *Pseudomonas syringae* effectors can alter chromatin structure to modulate plant innate immunity.

Key Words: *Pseudomonas*, Chromatin, Acetylation, Effectors, Immunity.

A bacterial effector that specifically suppressed MAMP-induced activation of the MAPKs, MPK4 and MPK11.

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Mitogen-activated protein kinases (MAPKs) regulate several cellular signalling pathways in plants. Exposure to potential pathogens or microbe-associated molecular patterns (MAMPs) induces the rapid activation of at least four MAPKs (namely MPK3, MPK6, MPK4 and MPK11), which then target downstream substrates to induce the appropriate defence reactions. MPK3 and MPK6 are associated with the signalling pathway dealing with positive regulation of the pathogen response. MPK4, on the other hand, acts in a second MAPK signalling branch that is so far linked to negative control of SA-regulated defence. MPK11 is highly homologous to MPK4, with some partially overlapping functions to MPK4. Several pathogen effector proteins have been shown to subvert antimicrobial defence responses by inactivating MAPKs. This includes the *Pseudomonas syringae* HopAI1, a phosphothreonine lyase, which irreversibly modifies the activation loop of MAPKs, thus preventing their phosphorylation and activation. However, such effectors can target all four MAMP-activated, and presumably other, MAPKs. We present here a *P. syringae* effector that specifically suppresses the MAMP-induced MPK4/MPK11 activation but not the MPK3/MPK6 pathway. Thus, this presumably represents a virulence function of this effector and would also imply that the MPK4/MPK11 must have positive signalling functions for defence activation. In view of the lethality or developmental defects of mpk3 mpk6 or mpk4 mpk11 double mutants, understanding the molecular mechanisms of MAPK-targeting effectors may aid the design of tools for interfering with specific MAPK pathways.

Key Words: defence, effector, MAMPs, MAPKs, virulence.

Natural diversity and functional requirements of TALEs in the *Xanthomonas oryzae* - rice interaction

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Transcription activator-like effectors (TALEs) from diverse plant-pathogenic *Xanthomonas* species are transcription factors which induce the expression of target genes in the host plant. TALE proteins bind to DNA via tandem repeats of typically 34 amino acids. Each repeat recognizes one base in the target DNA sequence via one precisely positioned amino acid of a repeat-variable diresidue (RVD). The simple and modular repeat architecture allows natural as well as artificial rearrangement of TALE repeats to generate TALEs with virtually any DNA-binding specificity.
Xanthomonas oryzae pv. *oryzae* (*Xoo*) and *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) are the causal agents of two devastating rice diseases worldwide. *Xoo* and *Xoc* strains typically carry large numbers of TALE genes (up to 26 per strain), but the overall TALE diversity, TALE evolution, and most TALE virulence targets are unknown. To gain insight into the evolution of TALE-based pathogenicity in *Xoo* and *Xoc* we compared and classified TALE gene repertoires and identified common TALE classes. Surprisingly, some TALEs from *Xoo* strains of different geographic origin induce the same or related plant genes indicating a convergent evolution towards important virulence targets. One example for such a target is the rice OsSWEET14 gene which encodes a sugar exporter. We demonstrated that only a defined class of five rice SWEET genes supports bacterial growth, indicating that all of these are sugar exporters. We analyzed whether specific promoter regions are required for TALE function. Using a collection of artificial TALEs that bound to various sequences in the OsSWEET14 promoter, we showed that TALEs are able to activate gene expression from different positions, but that activation efficiency is position dependent. These experiments give novel insights into the functional requirements of transcriptional activation by TALEs.

Key Words: *Xanthomonas*, TALE, rice, SWEET, transcription factor.

REFERENCES

BOCH, J.; SCHOLZE, H.; SCHORNACK, S.; LANDGRAF, A.; HAHN, S.; KAY, S.; LAHAYE, T.; NICKSTADT, A.; BONAS, U. (2009): Breaking the code of DNA binding specificity of TAL-type III effectors. Science 326 (5959), 1509-1512.
STREUBEL, J.; PESCE, C.; HUTIN, M.; KOEBNIK, R.; BOCH, J.; SZUREK, B. (2013): Five phylogenetically close rice SWEET genes confer TAL effector-mediated susceptibility to *Xanthomonas oryzae* pv. *oryzae*. New Phytologist 200 (3), 808-819.

Phenotypic heterogeneity and effector-mediated mechanisms of plant defence evasion in *Pseudomonas syringae*

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Genetic diversity is essential for the process of natural selection and adaptation. Individuals with particular genotypes that enable them to outgrow competitors, survive stresses and adapt to different environments will be selected. Whereas sexual reproduction and meiotic recombination are the main sources of variation in eukaryotes, mutation, recombination and horizontal transfer have been traditionally considered the sources of phenotypic variation in prokaryotes. Indeed, the process of host adaption in plant pathogenic bacteria is heavily determined by type III secretion systems and type III-secreted effectors, which have been mostly acquired by the pathogen through horizontal transfer events. Thus, the ability to suppress plant defences, and to evade their activation of the effector repertoire of a given pathogen is a major factor determining host specificity.
We are investigating the functional role of type III secreted effectors in *P. syringae*, their relationships regarding cross-suppression of plant defences, as well as novel mechanisms contributing to host adaptation. Our recent application of single-cell analysis methods such as flow cytometry and fluorescent microscopy to address these questions has lead as to join up those that have challenged the notion of bacterial population as clonal, and therefore phenotypically homogeneous. We have found phenotypic variation in a major virulence determinant, the Hrp type III secretion system of *P. syringae*, and have investigated its relevance in the process of host adaptation. We present our findings in this and other aspects of the process of effector-mediated adaptation to the host.

Key Words: phenotypic variation, host adaptation, defence suppression, T3SS, effector.

ABSTRACTS

POSTER PRESENTATIONS

P1. Insights into antimicrobial secondary metabolite production and regulation in *Pseudomonas corrugata* and *P. mediterranea*.

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The genome sequences of nine strains of the closely-related bacterial species *Pseudomonas corrugata* (*Pco*) and *Pseudomonas mediterranea* (*Pme*) provide new opportunities to investigate their lifestyle (Trantas et al. 2014). Both species are the causal agents of tomato pith necrosis, yet their genome analysis revealed the absence of a type III secretion system and of known type III effectors. They are also found in the soil of cultivated plants and are associated to the rhizosphere or as endophytes of many plants where they apparently do not cause any symptoms (Catara, 2007). The application of strains as biocontrol agents was thus explored for soil/root application in various host-pathogen interactions. Their antimicrobial activity has been mainly linked to the production of the cyclic lipopeptides (CLPs) corpeptins and cormycin (Licciardello et al., 2012). We hypothesized that the production of additional secondary metabolites might enhance competitiveness and biocontrol fitness. In each genome, different secondary metabolite gene clusters were identified, related to the production, secretion and regulation of at least three non-ribosomal peptides. Single clusters for the biosynthesis of polyketides, bacteriocins, siderophores, arylpolyenes, and hydrogen cyanide (HCN) were also discovered. A lantipeptide gene cluster was found in *Pme* genomes alone. A number of mutant strains (Quorum sensing, RfiA transcriptional regulator, corpeptin NRPS, and HCN synthesis cluster), were tested in vitro against Gram-positive and Gram-negative bacteria and fungi. We found that not only cyclic lipopeptides cormycin and corpeptins are produced by *Pco* and *Pme*, but also other diffusible molecules with antimicrobial activity and HCN inhibition of fungal growth and sporulation. PcoR and PmeR (the LuxR-cognate regulator proteins of the Quorum sensing systems) have a role in cormycin and corpeptin production, but RfiA seems key in the production of all antifungal diffusible metabolites except for HCN.

Key Words: *Pseudomonas*, antimicrobial gene clusters, CLPs, HCN, transcriptional regulators.

REFERENCES

CATARA (2007): *Pseudomonas corrugata*: plant pathogen and/or biological resource?. Molecular Plant Pathology, 8 (3), 233-244.
LICCIARDELLO, G. STRANO, C.P., BERTANI, I., BELLA, P., FIORE, A., FOGLIANO, V., VENTURI, V., CATARA, V. (2012): N-acyl-homoserine-lactone quorum sensing in tomato phytopathogenic *Pseudomonas* spp. is involved in the regulation of lipodepsipeptide production. Journal of Biotechnology, 159 (4), 274-282.
TRANTAS, E.A.; WARD, B.; WITEK, K.; LICCIARDELLO, G.; GOUMAS, D.E.; VAN OOSTERHOUT, C.; GUTTMAN, D.E.; CATARA, V.; SARRIS, P.F. (2014): Comparative genomics of multiple strains of *Pseudomonas corrugata* and *Pseudomonas mediterranea*. XVI International Congress on Molecular Plant-Microbe Interactions, 6-10 July, Rhodes, Greece.

P2. Dynamic evolution and role of the Pac_ICE in *Pseudomonas syringae* pv. *actinidiae*

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A polymorphic region with similarity to the integrative and conjugative element (ICE) PPHGI-1 was identified in the aggressive form of *Pseudomonas syringae* pv. *actinidiae* (*Psa*-V or *Psa* 3) - the causal agent of the pandemic canker disease affecting kiwifruit. This region was thought to be present only in strains responsible for the 2008 outbreak and has been implicated in heightened virulence. Subsequently three different ICEs have identified among the *Psa*-V strains: Pacific (Pac_ICE1), Mediterranean (Pac_ICE2) and Andean (Pac_ICE3). They are syntenous though divergent at the nucleotide level (75% nucleotide identity), but share a region of 20 Kb which differs by just four SNPs. The conserved region, referred to as enolase region, has an unknown function, but it carries genes hypothesized to be involved in the interaction between host and pathogen (McCann et al., 2013; Butler et al., 2013).
Bioinformatics analyses reveal the spread of the enolase region only among plant pathogens belonging to the *Pseudomonas syringae* species, suggesting a shared mechanism for the species in plant pathogen interaction that could lead to the development of the disease.
The identification of additional ICEs in the genome of several *Psa* isolated in China (both belonging or not to the *Psa*-V clade), shapes the evolution of the ICEs itself which doesn't follow the phylogeny of the strains.
A mutagenesis approach, consisting in the creation of in-frame deletion mutants and their in planta analysis, is currently being used to understand the role of the enolase region in plant-pathogen interactions.

Key Words: Integrative Conjugative Element, Virulence Determinants, Plant-Pathogen Interaction, Epiphytic Fitness.

REFERENCES

MC CANN, H. C., RIKKERINK, E. H. A., BERTELS, F., FIERS, M., LU, A., REES-GEORGE, J., et al. (2013). Genomic analysis of the kiwifruit pathogen *Pseudomonas syringae* pv. *actinidiae* insights into the origins of an emergent plant disease. PLoS Pathogens, 9, e1003503.
BUTLER,M. I., STOCKWELL, P. A., BLACK,M.A., DAY, R. C., LAMONT, I. L., & POULTER, R. T. M. (2013). *Pseudomonas syringae* pv. *actinidiae* from recent outbreaks of kiwifruit bacterial canker belong to different clones that originated in China. PLoS ONE, 8, e57464.

P3. Biochemical and in planta characterization of the compartment-specific enzymatic activities of the extracelluar enzymes, LscB and LscC, of the plant pathogen *Pseudomonas syringae*

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Pseudomonas syringae pv. *glycinea* PG4180, the causative agent of bacterial blight of soybean plants, possesses several virulence factors, one of them being the synthesis of exopolysaccharides. One of them, levan, is a polymer of fructose, which is synthesized from sucrose by two highly similar enzymes (LscB and LscC) levansucrases. Due to the remarkably different sub-cellular localization of the two enzymes in *P. syringae*, and an indepth enzymatic characterization of LscB and LscC is needed. We hypothesize that both enzymes might conduct different compartment-specific reactions. This assumption is substantiated by the finding that Lsc generally is known to catalyze three divergent reactions: a) cleavage of sucrose into glucose and fructose; b) polymerization of fructosyl residues to form levan; and c) depolymerization of levan to yield free fructosyl residues. To date it is unclear whether both, LscB and LscC, conduct all three reactions at similar rates and efficiencies. LscB (M5) and LscC (M3) mutants have been generated by homolgous recombination in order to study the role of LscB and LscC enzymes individually, both in vivo and in plant. Filter-sterilized supernatants of the wild type PG4180, M5 and M3 cultures were used for enzyme purification by gel-filtration chromatography (SEC). The km value of each of the enzymes is determined under different conditions i.e pH and temperature. In addition, the levan degrading activities of both the enzymes are tested. Furthermore, in planta growth of wild type PG4180, M5 and M3 mutants was evaluated on soybeans (*Glycine max* (L.) Merr.) cultivar Maple Arrow. Soybean seedlings were germinated and grown in the greenhouse for three to four weeks prior to the growth assays. For spray inoculation, the cells were adjusted to an OD600 of 0.1 (corresponding to approximately 10^7 CFU/ml) and applied to the leaves with an airbrush (~8 psi) until the leaf surfaces were uniformly wet. Subsequently, inoculated plants were grown in the greenhouse (19-21°C), and survival and growth of bacterial strains was monitored by removing random leaf samples at 1-14 days post inoculation. Bacterial counts (CFU/g fresh weight) were determined by plating dilutions of leaf homogenate onto MG agar and counting of fluorescent colonies after incubation for 96 h.

P4. Role in virulence of a *Pseudomonas savastanoi* pv. *savastanoi* NCPPB 3335 genomic region shared with other pathogens of woody host

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The genome of the olive tree pathogen *Pseudomonas savastanoi* pv. *savastanoi* (*Psv*) NCPPB 3335 (58.1% G+C) encodes a region of about 15 kb, named VR8 (60.4% G+C), which is absent in all sequenced *Pseudomonas syringae* strains infecting herbaceous plants (non-lignified), but shared with other *P. syringae* pathovars infecting woody hosts (lignified). RT-PCR analysis of the VR8 genes revealed the existence of 4 possible operons and three genes independently transcribed, two of which (AER-1899 and AER-1907) are putative transcriptional regulators. Here we demonstrate that the *antABC* and *catBCA* operons are involved in the degradation of anthranilate and catechol, respectively. The oxygenase activity associated with the AER-1901/2/3 operon is capable of converting indole into indigo when overexpressed in bacterial cultures. On the other hand, the coding sequence AER-1900 is annotated as an aerotaxis receptor, while the AER-1904/5 operon shows homology to genes related with the metabolism of aromatic compounds. RT-qPCR and β-galactosidase assays of a *lacZ* fusion to the *antABC* promoter showed that both anthranilate and 6-chloroanthranilate induce the expression of the *antABC* operon. In addition, anthranilate also induces transcription of the *catB*. To analyse the role in virulence of the VR8, we constructed several knockout mutants affected in this region. The volume of the knots developed by the *antABC*, *catBCA*, AER-1900, AER-1901/2/3 and AER-1904/5 mutants on non-lignified olive plants was similar to those developed by the wild-type strain, whereas the *antABC*, *catBCA*, AER-1901/2/3 and AER-1904/5 mutants generated knots significantly smaller than those induced by the wild-type strain on woody plants. Moreover, the *catBCA*, AER-1901/2/3 and AER-1904/5 operons, as well as the AER-1900 gene, are necessary for full fitness of the pathogen on lignified olive plants, while only AER-1901/2/3 is also essential for fitness on non-lignified plants. These results suggest a possible role of VR8 region in the metabolism of aromatic compounds linked to lignin synthesis/degradation.

Key Words: aromatic compounds, woody host, virulence

P5. Biosynthesis and regulation of mangotoxin production in *Pseudomonas syringae*

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The mangotoxin is a key factor in virulence of *Pseudomonas syringae* pv. *syringae* strains which cause apical necrosis of mango trees. This antimetabolite toxin is an oligopeptide that inhibits ornithine N-acetyl transferase, a key enzyme in the biosynthesis of ornithine and arginine. In this study random mutagenesis led to the identification of three gene clusters that affect mangotoxin biosynthesis. These are the *gacS*/*gacA* genes. This operon that harbors four genes *mgoBCAD* and the *mbo* operon, which is specific and essential for mangotoxin biosynthesis and is composed by six genes *mboABCDEF*. mbo operon heterologous expression in non-producing *P. syringae* strains led in mangotoxin production. Transcriptional analyses by qPCR and promoter reporter fusions revealed that *mbo* expression is regulated by both *gacS*/*gacA* and *mgo* genes. Also, expression of the *mgo* operon was shown to be regulated by *gacS*/*gacA*. Heterologous expression under the native promoter of the *mbo* operon resulted in mangotoxin production in non-producing *P. syringae* strains, but not in other *Pseudomonas* species. The introduction of the *mbo* and *mgo* operons in nonproducing *P. protegens* Pf-5 did not confer mangotoxin production but did enhance transcription of the mbo promoter. In this study we also determined the evolutive history of the *mbo* operon. Phylogenetic analyses using partial sequences from housekeeping genes differentiated three groups within genomospecies 1. All of the strains containing the *mbo* operon clustered in groups I and II, whereas those lacking the operon clustered in group III. We concluded that the mbo operon could be acquired horizontally and only once by the ancestor of groups I and II. Mangotoxin biosynthesis is governed by the *mbo* operon, and both *mbo* and *mgo* operons are under the control of the *gacS*/*gacA* two-component system, and finally, the MgoA product could acts as a positive regulator of mangotoxin biosynthesis.

Key Words: Antimetabolite toxin, *mgo* operon, *mbo* operon, GacS/GacA, Plant-microbe interaction.

P6. The restoration of *cmaL* to *Pto* DC3000D28E derivatives with minimal effector sets reveals the essential role of coronatine in disease symptom formation in *Nicotiana benthamiana*

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Pseudomonas syringae pv *tomato* DC3000 causes bacterial speck disease in tomato and *Arabidopsis*, and also *Nicotiana benthamiana* when it carries a deletion of the avirulence gene *hopQ1*-1. Disease caused by DC3000 is due to suppression of plant defense responses by ~30 effectors delivered into plant cells via the type III secretion system, and the phytotoxin coronatine (COR). COR is formed by a linkage of two molecules, coronafacic acid (CFA) and coronamic acid (CMA)1.
We recently identified that the DC3000 *cmaL* gene is required for the biosynthesis of CMA2. We reported previously the construction of a "nearly effectorless" mutant of DC3000, DC3000D28E, that is defective in the production of 28 type III secretion system effectors (T3Es)3. D28E carries a deletion of *cmaL*, but encodes a functional *cfa* operon. With available genetic tools to reintegrate *cmaL* to genomic locations and restore COR production, D28E provides a clean platform with minimal effector interplay to study the action of COR.
We restored *cmaL* to D28E derivatives containing minimal effector sets, and studied their ability to cause disease symptoms and growth in *N. benthamiana*. We observed that *cmaL* caused some symptoms in the absence of T3Es or the type III secretion system during dip-inoculation of plants. The intensity of symptoms, however, increased with the number of T3Es pre-sent in the background. The strain with fewest T3Es and the most striking gain-of-symptoms was D28E+*avrPtoB*+*hopM1*::*cmaL*. We observed that COR did not contribute to growth of different strains during dip- or syringe- inoculation of plants, nor did *cmaL* provide any benefit for bacterial entry during dipping. Symptoms attributable to COR ranged from discrete chlorotic spots to intense necrosis depending on levels of inoculum. Chlorotic spots were found to be associated with well-established bacterial colonies. We concluded that the primary role of COR is in symptom development.

Key Words: Coronatine, *cmaL*, DC3000D28E, *Nicotiana benthamiana*

REFERENCES

1. GENG X, JIN L, SHIMADA M, KIM MG, MACKEY D. (2014): The phytotoxin coronatine is a multifunctional component of the virulence armament of *Pseudomonas syringae*. Planta, 240(6): 1149-1165.
2. WORLEY JN, RUSSELL AB, WEXLER AG, BRONSTEIN PA, KVITKO BH, KRASNOFF SB, MUNKVOLD KR, SWINGLE B, GIBSON DM, COLLMER A. (2013): *Pseudomonas syringae* pv. *tomato* DC3000 CmaL (PSPTO4723), a DUF1330 family member, is needed to produce L-allo-isoleucine, a precursor for the phytotoxin coronatine. Journal of Bacteriology, 195(2): 287-296.
3. CUNNAC S, CHAKRAVARTHY S, KVITKO BH, RUSSELL AB, MARTIN GB, COLLMER A. (2011): Genetic disassembly and combinatorial reassembly identify a minimal functional repertoire of type III effectors in *Pseudomonas syringae*. Proceedings of the National Academy of Sciences U S A, 108(7): 2975-2980.

P7. Temporal and structural stability of virulence plasmids of *Pseudomonas syringae* is enhanced by multiple toxin-antitoxin systems

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Native plasmids of *P. syringae* often carry virulence genes, and greatly facilitate adaptation by gene trading among bacterial populations. Plasmids are extremely stable despite extensively sharing replication regions, containing repeated sequences or, for some, lacking any obviously selectable genes. To investigate the evolutionary forces driving plasmid dynamics and survival in *P. syringae*, and to better evaluate their contribution to pathogenicity, we undertook the identification and functional characterization of stability determinants of the completely sequenced plasmids of *P. syringae* pv. *savastanoi* NCPPB3335 (pPsv48A, 73 kb; pPsv48B, 45 kb, and pPsv48C, 42 kb), all of which contain putative virulence genes. From the 11 determinants that significantly increase the stability of an artificial construct, and because they conferred very high stability, we selected three toxin-antitoxin systems each from pPsv48A and pPsv48C for further analyses and as tools for plasmid curing. Complementation analyses of NCPPB3335 with the three cloned antitoxins from pPsv48A increased its loss by only a factor of ten, indicating the existence of other determinants of functional relevance. In turn, complementation with the three antitoxins from pPsv48C allowed the emergence of derivatives of this plasmid containing deletions of variable length. These were mediated by one-ended transpositions of IS801 and maintained thanks to a previously cryptic replicon adjacent to this element. The generation of new plasmid architectures mediated by IS801 also occurred with pPsv48A, suggesting it might be a universal phenomenon in *P. syringae*. Additionally, the toxin-antitoxin genes PSPSV_C0050/0051 also acted limiting the occurrence in pPsv48C of a deletion of 8.3 kb mediated by MITEPsy2. Using *sacB*-based negative selection and antitoxin genes, we obtained a plasmid-free derivative of NCPPB3335, whose virulence is being evaluated. Our results suggest that the multiple stability determinants often found in native plasmids contribute to survival by vertical transmission and to preserve structural integrity, also contributing to maintenance of virulence determinants.

Key Words: evolution of virulence, replication, pathogenicity, horizontal transfer

P8. Indole-3-acetic acid in *Pseudomonas savastanoi* pv. *savastanoi* NCPPB 3335; new findings and roles

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The production of the phytohormone indole-3-acetic acid is widespread among plant-associated bacteria, and it is especially important for the effective development of disease symptoms by the tumour-inducing pathogen *Pseudomonas savastanoi* pv. *savastanoi*. Several IAA biosynthetic pathways have been described in phytopathogenic bacteria, but the best-characterised one is the indole-3-acetamide pathway. In this pathway, the genetic determinants involved in the conversion of tryptophan (Trp) to IAA are Trp monooxygenase (encoded by the *iaaM* gene), which converts Trp to indoleacetamide (IAM), and IAM hydrolase (encoded by the *iaaH* gene), which catalyses the conversion of IAM to IAA. A phylogenetic analysis of the nucleotide sequence of the *iaaM*/*iaaH* operon showed that one of the two operons encoded by *Pseudomonas savastanoi* pv. *savastanoi* NCPPB 3335, *iaaM*-1/*iaaH*-1, is most likely, horizontally transferred among bacteria of the *Pseudomonas syringae* complex. Moreover, we also demonstrated that the biosynthesis of the phytohormone, virulence and full fitness of this olive pathogen only depend on the functionality of the *iaaM*-1/*iaaH*-1 operon. In contrast, the *iaaM*-2/*iaaH*-2 operon, which carries a 22-nucleotide insertion in the *iaaM*-2 gene, does not contribute to the production of indole-3-acetic by this bacterium. Interestingly, a small amount of indole-3-acetic was still detected in the culture supernatants of a double mutant affected in both *iaaM*/*iaaH* operons, suggesting that a different pathway might also contribute to the total pool of the phytohormone produced by this pathogen. Additionally, we showed that exogenously added indole-3-acetic negatively and positively regulates the expression of genes related to the type III and type VI secretion systems, respectively. Together, these results suggest a role of indole-3-acetic acid as a signalling molecule in *Pseudomonas savastanoi* pv. *savastanoi*.

Key Words: *Pseudomonas savastanoi*, olive knot disease, indol-3-acetic acid, T3SS, T6SS.

REFERENCES

ARAGON, I. M.; PEREZ-MARTINEZ, I.; MORENO-PEREZ, A.; CEREZO, M.; RAMOS, C. (2014). New insights into the role of indole-3-acetic acid in the virulence of *Pseudomonas savastanoi* pv. *savastanoi*. FEMS Microbiology Letters, 356(2), 184-192.

P9. Transcript comparison of *Pseudomonas syringae* pv. *actinidiae* genes expressed in vitro and in planta

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Domestication of kiwifruit began in the 1930s with commercial plantings of *Actinidia deliciosa* 'Hayward', followed by release of several *A. chinensis* cultivars, most notably 'Hort16A' in the 1990s. This resulted in substantial plantings around the world of largely clonal material possessing little genetic diversity. *Pseudomonas syringae* is a widespread species complex that comprises plant epiphytes and pathogens and has also been found in non-agricultural environments such as waterways. Each pathovar of *P. syringae* posseses a narrow host range related to the specific effector and secondary metabolite profile is possesses. *P. syringae* pv. *actinidiae* (*Psa*) caused two outbreaks of canker disease in Japan and Korea in the mid 1980s and 1990s respectively. However these disease outbreaks did not spread from their country of origin. In 2008 a particularly virulent strain of *Psa* was reported in Italy and it quickly decimated plantings of *A. chinensis*. Subsequently the disease was reported in other kiwifruit growing regions around the world such as New Zealand, China and Chile. Based on SNP analyses of two circularized and 30 draft genomes, *Psa* was shown to be comprised of four distinct clades exhibiting negligible within-clade diversity [3]. *Psa* has an overall clonal population structure, however genomes carry a marked signature of within-pathovar recombination. These data are consistent with disease arising by independent samplings from a source population. Genomic comparisons show a dynamic genome with evidence of positive selection on type III effectors and other candidate virulence genes. Each clade has a surprisingly variable accessory genome with different complements of genes encoding effectors and toxins. Many of these genes are coded on active mobile genetic elements. While bioinformatic analysis has identified genes that might be unique to the recent outbreak clade little is known about the expression of these and other genes that might have a crucial role in pathogenicity. To gain additional information about the role of these genes we have carried out transcriptome analysis of *Psa* growth both in vitro on minimal media and during the early stages of kiwifruit infection, and compared the expression of genes identified as potential pathogenicity factors.

P10. Over-expression of virulence inhibiting peptides in *Nicotiana tabacum* plants as a tool to control *Pseudomonas syringae* pv. *tabaci*

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The use of antimicrobial peptides (AMPs) to control phytopathogenic bacteria and fungi has been suggested as a potential alternative to conventional pesticides and antibiotics (Montesinos, 2007). Transgenic plants expressing AMPs could represent a good strategy to fight pathogens in both animal and plant hosts, although some concern still exist about several their intrinsic drawbacks (Keymanesh et al., 2009). Conversely, the design and use of innovative oligopeptides targeting Type Three Secretion System (T3SS) of *Pseudomonas savastanoi* pv. *nerii* has been previously showed to inhibit the development of the disease in its host *Nerium oleander*, without any toxic impact on plants (Cerboneschi et al., 2012). In this work, we describe the stable transformation and expression in *Nicotiana tabacum* plants of two of these short peptides, 17 and 27 aminoacids long, named AP17 and LI27, respectively. The transformation of Tobacco plants was performed using *Agrobacterium* EHA105, carrying the pCambia 1305.2Δ*gus* binary vector, containing AP17 or LI27 sequences downstream the signal peptide GRP to target peptide delivery to apoplast (Biancalani et al., 2014). Transgenic lines were scored for antibiotic resistance on Linsmaier and Skoog medium containing 50 mg/l hygromycin, and then for AP17 or Li27 expression. Transgenic lines expressing high levels of each peptide were selected, and then *in vitro* inoculated with *P. syringae* pv *tabaci* ATCC 11528 (*Pstab*). While control untrans-formed plants were severely affected by *Pstab* infection, AP17 and Li27 transgenic plants showed faint or no symptoms, and the bacterial growth was severely impaired to reach zero level in few days after inoculation. This data demonstrated that constitutively ex-pressed virulence inhibiting peptides could represent a promising strategy to control bacterial diseases of plants. Prelimary experiments are ongoing about the stable transformation and expression of *Actinidia chinensis* with peptides targeting T3SS of *Pseudomonas syringae* pv. *actinidiae*.

Key Words: AMPs, *Nicotiana tabacum*, transgenic plants, T3SS, *Pseudomonas syringae*.

REFERENCES

BIANCALANI, C.; CERBONESCHI, M.; MACCONI, S.; MONCELLI, M.R.; SMEAZZETTO, S.; BIRICOLTI, S.; BOGANI, P.; TEGLI, S. (2014): Peptides as inhibitors of type three secretion system for ecofriendly control of bacterial diseases of plants. Environmentally loyal plant protection: from nano- to field-scale, XX SIPAV Congress, p. 37, Pisa, September 22-24, 2014.
CERBONESCHI, M.; BRITTOLI, A.; ARRIGONI, E.; CAPARROTTA, S.; CIOLLI MATTIOLI, C.; COLOMBI, E.; BIANCALANI, C.; MACCONI, S.; TEGLI, S. (2012): Protein-protein interaction model: new strategies for bacterial diseases control. Proceedings of the 3rd International Workshop on Expression, Structure and Function of Membrane Proteins, p. 24, Florence, September 23-27, 2012.
KEYMANESH, K.; SOLTANI, S.; SARDARI, S. (2009): Application of antimicrobial peptides in agriculture and food industry. World Journal of Microbiology and Biotechnology, 25 (6), 933-944.
MONTESINOS, E. (2007): Antimicrobial peptides and plant disease control. FEMS Micro-biology Letters, 270 (1), 1-11.

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P11. Epigallocatechin gallate and other polyphenols extract from plant biomass for environment-friendly control of *Pseudomonas syringae* diseases

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Although bacterial diseases of plant are relatively few in comparison to those caused by viruses and fungi, they annually cause heavy economic losses all over the world. Damages caused on vegetable and fruit crops may be destructive under adverse environmental conditions or in case of quarantine pathogens. The control of bacterial diseases of plants is worldwide a considerable challenge in the agriculture practice, because of the limited availability of bactericides, that are represented mainly by copper and antibiotics, the latter not allowed in EU for plant protection. In order to meet the needs related both to the productivity of agro-industry and to the protection of the environment, alternatives to the use of copper compounds against phytopathogenic fungi are already under study, possibly able to block their ability to harm the host instead that their viability. Among the few synthetic compounds evaluated until now on phytopathogenic bacteria there are salicylidene acylhydrazides, targeting the Type Three Secretion System (T3SS) of *Erwinia amylovora* (Yang et al., 2014). Similarly, against the T3SS of this bacterium several phenolic compounds and their derivatives were shown to have inhibitory activity (Khokhani et al., 2013). In this work *P. savastanoi* pv. *nerii*, *P. syringae* pv. *tabaci* and *P. syringae* pv. *actinidiae* were used as model systems to evaluate the use of polyphenol-based molecules extracted from agricultural plant biomass as T3SS inhibitors, together with epigallocate-chin-3-gallate (EGCG) which is the main phenol present in green tea. The effectiveness of these polyphenols was demonstrated by using a green fluorescent protein (GFP) reporter system to monitor T3SS expression in these phytopathogenic bacteria, and by *in vitro* and *in vivo* pathogenicity trials on their host plants and HR on tobacco. Furthermore, gene expression studies were also carried out to unveil the bacterial pathways altered by these phenolic extracts.

Key Words: T3SS, *P. syringae*, polyphenols, phenolic extracts, EGCG, green tea.

REFERENCES

KHOKHANI, D.; ZHANG, C.; LI, Y.; WANG, Q.; ZENG, Q.; YAMAZAKI, A.; HUTCHINS, W.; ZHOU, SS.; CHEN, X.; YANG, CH. (2013): Discovery of plant phenolic compounds that act as Type III Secretion System inhibitors or inducers of the Fire Blight pathogen, *Erwinia amylovora*. Applied and Environmental Microbiology, 79 (18), 5424-5436.
YANG, F.; KORBAN, S.S.; PUSEY, P.L.; ELOFSSON, M.; SUNDIN, G.W.; ZHAO, Y. (2014): Small-molecule inhibitors suppress the expression of both type III secretion and amylovoran biosynthesis genes in *Erwinia amylovora*. Molecular Plant Pathology, 15 (1), 44-57.

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P12. High-frequency transfer of a multiresistant mobile genetic element among strains of *Pseudomonas syringae*

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Chemical treatments with copper and/or streptomycin is a main strategy for field management of phytopathogenic bacteria, although resistance to one or both has been repeatedly found in *P. syringae* and other species, and often associated to mobile genetic elements. Generally, resistance is linked to an excessive use of these compounds and strategies to improve control foster an adequate planning of treatments. *P. syringae* pv. *syringae* UPN800 is pathogenic to beans and able to transfer simultaneously copper and streptomycin resistance to *P. syringae* pv. *phaseolicola* 1448A at frequencies of 10^{-1}-10^{-4} (transconjugants per recipient cells), both in culture medium and in planta. Transfer of e800 probably occurs by conjugation, and recipient cells of 1448A can in turn transfer it to strains of *P. syringae* pvs. *syringae* and *savastanoi*. Genome sequencing and PCR analyses showed that resistance genes were associated to a fragment of approximately 272 kb (e800), which by qPCR analyses appears to be in single copy in the harboring bacteria; however, e800 is probably a plasmid because it appears as an extrachromosomal band in DNA hybridizations of plasmid profile gels. Among its approximately 340 CDSs, e800 contains Tn5393 (Str^R) and homologs of different genes and operons for resistance to arsenic, cadmium, copper, lead, mercury, and nickel. Indeed, transconjugants of strain 1448A containing e800 showed increased MIC values on solid media supplemented with $CuSO_4$, $HgCl_2$, $NiCl_2$, $NaAsO_2$ and/or streptomycin. In Blast comparisons, and among others, e800 shows significant similarity and collinearity with plasmid pSTY from *Pseudomonas* sp. VLB120 and a chromosomal genetic island from the human isolate *P. putida* H8234. Our results indicate that multiresistance MGEs from environmental or human sources can readily be dispersed among populations of plant pathogenic bacteria, and that failure of chemical control methods can be the result of selection for resistance to common polluting metals.

Key Words: copper, streptomycin, heavy metals

P13. Functional analysis of *Pseudomonas savastanoi* pv. *savastanoi* type III secretion system effector families HopAF and HopAO

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Pseudomonas savastanoi pv. *savastanoi* (*Psv*) NCPPB 3335 is a tumor-inducing pathogen of *Olea europaea* causing olive knot disease. Bioinformatics analysis of the draft genome sequence of NCPPB 3335 allowed identification of 34 putative type III secretion system (T3SS) effector (T3E) proteins in this pathogen. Moreover, complete sequencing of the three-plasmid complement of this strain revealed that the genes encoding two of these putative T3Es are located on plasmids: *hopAF1* (pPsv48A), which is included into a transposon and shows a chromosomally encoded gene ortholog *(hopAF1-2*), and *hopAO1* (pPsv48B). NCPPB 3335 also encodes HopAO2, which harbors a similar protein tyrosine phosphatase (PTP) domain than of HopAO1. Phylogenetic analysis of the HopAF and HopAO families revealed that these T3Es are widely distributed among strains of the *P. syringae* complex. Translocation, expression and functional analysis of HopAF1, HopAF1-2, HopAO1 and HopAO2 validated all these proteins as members of the NCPPB 3335 T3E repertoire. Artificial inoculations of olive plants with NCPPB 3335 derivatives cured of plasmids pPsv48A or pPsv48A and pPsv48B previously showed that these two plasmids are necessary for full virulence and for the development of mature xylem vessels within the knots. The role of these T3Es in the interaction of NCPPB 3335 with olive plants and in the interference of the plant immune innate responses is currently under evaluation, as well as the analyses of their subcellular localization.

Key Words: HopAF, HopAO, T3SS, *Pseudomonas savastanoi* pv. *savastanoi*, olive knot disease, plant responses.

P14. Suppression of HopZ-effector triggered defence responses

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Most of the Type III Effectors (T3E) from plant pathogens can suppress plant defence responses. The interactions between the plant and the pathogen, as depicted in the zig-zag model proposed by Jones and Dangl in 2006, are believed to drive the co-evolution of bacterial effectors and plant resistance. Nevertheless, evolution models for effector families rarely take into account that selective pressure against Effector-Triggered Immunity (ETI) in a given host, may be compensated by an ETI-suppressing effector encoded by the same strain.
The *Pseudomonas syringae* HopZ effector family has been well characterized from an evolutionary point of view. It is one of the most diverse, and displays a high rate of horizontal transfer between pathovars. Changes in the sequence of the different members of the HopZ family have also been proposed to play a role in host adaptation, by preventing detection within their respective hosts, without losing out on their virulence activities, in a process called pathoadaptation (Ma et al., 2006).
Here we present how different HopZ effectors do not trigger immunity in their respective pathosystems. However, expression of these effectors from heterologous strains does trigger immunity within the same plant hosts, indicating that the hosts can detect their presence and activate a defence response. Thus, the strains naturally encoding the HopZ effectors can prevent HopZ-triggered immunity, likely through the action of a cross-suppressing effector. We identify the effectors suppressing HopZ3-triggered immunity, and propose cross-suppression of ETI as an alternative force driving evolution of the HopZ family.

Key Words:evolution, ETI, host adaptation, defence suppression, T3SS, effector.

REFERENCES

MA, W.; DONG, F. F. T.; STAVRINIDES, J.; GUTTMAN, D. S. (2006): Type III effector diver-sification via both pathoadaptation and horizontal transfer in response to a coevolutionary arms race. PLoS Genetics, 2 (12), 2131-2142.

P15. Far beyond HopQ1 in the interaction between *Nicotiana* spp. and *Pseudomonas syringae sensu lato*

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HopQ1 is an effector secreted by some strains of *P. syringae sensu lato* through the Type Three Secretion System, and which is suggested to be involved in host range determination (Ferrante et al., 2009). To definitely unveil its role in pathogenesis, data from increasing *P. syringae sensu lato* genomes is pivotal, as well as to have several model plants, whose genomic data are accessible or easily achievable, and amenable to molecular techniques (i.e. transformation, targeted mutagenesis and gene-silencing). Besides *Arabidopsis thaliana*, the most popular model plants for many *P. syringae* bacteria also included *Nicotiana benthamiana*, *N. tabacum* and Tomato. The development of similar model pathosystems is particularly useful to speed up studies on *P. syringae* diseases of woody plants, which are often less prone than herbaceous species to genetic manipulation and *in vitro* culture. In this work *N. langsdorffii* was used for the first time as a model species, challenged by *P. savastanoi* pv. *nerii* Psn23, a strain missing the gene coding for the effector HopQ1. This feature was here demonstrated to be mainly associated with pv. *nerii* strains rather than with those from *savastanoi* or *fraxini* pvs. Typical hyperplastic symptoms were induced by Psn23 wild type on *N. langsdorffi*, on which the bacterium multiplied comparably to its host *Nerium oleander*. Similarly, when overexpressed by Psn23, HopQ1 was demonstrated to be a virulence factor in both *N. oleander* and *N. langsdorffii*. Conversely on *N. glauca*, which has an opposite hormonal profile compared to *N. lansgdorffi* (Fuoco et al., 2013), no symptoms and bacterial growth were ever observed after inoculation of both Psn23 wild type or overexpressing *hopQ1*. When *N. langsdorffii* hormone profile was altered by stable expression of GR and *rolC* genes (Giannarelli et al. 2010), its master role in the fate of HopQ1 interaction with Psn23 was confirmed.

Key Words: HopQ1, T3SS, *N. langsdorffii*, plant-host interactions, hormone profile, *P. syringae*.

REFERENCES

FERRANTE, P.; CLARKE, C.R.; CAVANAUGH, K.A.; MICHELMORE, R.W.; BUONAURIO, R.; VINATZER, B.A. (2009): Contributions of the effector gene *hopQ1*-1 to differences in host range between *Pseudomonas syringae* pv. *phaseolicola* and *P. syringae* pv. *tabaci*. Molecular Plant Pathology, 10(6), 837-842
FUOCO, R.; BOGANI, P.; CAPODAGLIO, G.; DEL BUBBA, M.; ABOLLINO, O.; GIANNAREL-LI, S.; SPIRITI, M.M.; MUSCATELLO, B.; DOUMETT, S.; TURETTA, C.; ZANGRANDO, R.; ZELANO, V.; BUIATTI, M. (2013): Response to metal stress of *Nicotiana langsdorffii* plants wild-type and transgenic for the rat glucocorticoid receptor gene. Journal of Plant Physiology, 170, 668-675.
GIANNARELLI, S.; MUSCATELLO, B.; BOGANI, P.; SPIRITI, M.M.; BUIATTI, M.; FUOCO, R. (2010): Comparative evaluation of the determination of some phyto-hormones in plants by GC-MS and HPLC-MS. Analytical Biochemistry, 398, 60-68.

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P16. Role of gene silencing in the regulation of R genes involved in the plant response against *Pseudomonas syringae*.

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Plants are under different types of stress that can be categorized according to their origin into biotic (pathogens) and abiotic (physical and chemical variations).
In plants, there are two main types of noncoding small RNA molecules, classified as microRNAs (miRNAs) and small interfering RNAs (siRNAs), which differ in their biogenesis and mode of action, but share similar sizes (20-24 nt). The precursors of these small RNAs, are processed by Dicer-Like RNase III (dcl) proteins present in *Arabidopsis thaliana*, and can act as negative regulators of gene expression, and are involved in a vast array of plant processes, including plant development, genomic integrity or response to stress. Regulation carried out through these small RNAs can occurs at transcriptional level (TGS) or at post-transcriptional level (PTGS).
In last years, the role of gene silencing in the regulation of genes related to the plant defence response against bacterial pathogens is becoming clearer.
We have carried out comparisons between the expression profiles of different mutants affected in gene silencing, and plants challenged with *Pseudomonas syringae* pathovar tomato DC3000. These comparisons has led us to identify a set of R genes of unknown functions, belonging to the TIR-NBS-LRR gene family, that are expressed differentially in both conditions. Plants carry different R (resistance) genes that encode proteins capable of directly or indirectly detecting bacterial effector proteins, triggering a strong defence response known as the effector-triggered immunity or ETI. Many R proteins present NBS-LRR (nucleotide-binding site and leucine rich repeats) domains. Bacterial effector proteins are directly injected into the plant host cell cytosol through the type III secretion systems (T3SS), where they can suppress the defence response triggered upon pathogen perception.
Using different bioinformatics tools, we have found a miRNA* that could by responsible for the regulation of the expression levels of these R genes, through the generation of siRNAs. In addition we identify one of these genes as a negative regulator of defence response against *Pseudomonas syringae*.

Key Words: Gene silencing, miRNA*, *Pseudomonas syringae*, *Arabidopsis*, R genes.

REFERENCES

LI, F.; PIGNATTA, D.; BENDIX, C.; BRUNKARD, JO.; COHN, MN.; TUNG, J.; SUN, H.; KU-MAR, P.; BAKER, B. (2012): MicroRNA regulation of plant innate immune receptors. Proc Natl Acad Sci USA, 31;109(5): 1790-5.

P17. *Pseudomonas syringae* triggered reduction of host histone H3K9 acetylation in Arabidopsis is type III effector driven and may involve histone deacetylase HDA5

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Pseudomonas syringae DC3000 employs a type III secretion system (T3SS) to inject effector proteins (T3Es) into plant cells and cause disease. We have been interested in determining the extent that *P. syringae* T3Es are involved in modulating host chromatin and thereby gene expression of immunity-related genes to favor pathogenesis. We have found a rapid deacetylation of host histone H3 lysine 9 (H3K9) in response to DC3000 but not to a T3SS defective Δ*hrcC* mutant. Using Chromatin immunoprecipitation assays we found reduced H3K9 acetylation along a subset of immunity-related genes in only the DC3000 infected plants and this correlated with decreased gene expression. To determine which T3Es are involved in the deacetylation, we analyzed plants infiltrated with poly-effector mutants lacking different combinations of T3E genes. Immunoblot analysis showed no deacetylation in plants infiltrated with a mutant lacking most of the T3Es, affirming a T3E role in deacetylation. We have also focused on determining which host proteins participate in the deacetylation process. Since H3K9 deacetylation could be caused by downregulation of histone acetyltransferase(s) (HAC(s)) or upregulation of histone deacetylases (HDAC(s)), we analyzed transcriptional changes of HACs and HDACs in *P. syringae*-exposed plants, and found at least one HDAC, HDA5, to be upregulated in plants exposed to DC3000 compared to those exposed to Δ*hrcC*. We are currently determining if HDA5 possesses any role in deacetylating H3K9 along immunity-related genes.

Key Words: effector biology, plant immunity.

P18. *Pseudomonas syringae* and other plant pathogens in lichen-associated microbiomes

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Although generally described as bipartite mutualistic associations of a fungus and an alga or cyanobacterium, lichens are also known to harbour species-specific communities of endolichenic bacteria, typically dominated by Proteobacteria. Among lichen-associated bacteria, revealed by both metagenomic and culture-based studies, are several known or suspected plant pathogens, including *Pseudomonas syringae*, *Erwinia persicina*, *Xanthomonas campestris*, and *Burkholderia glathei*.
Analysis of 30,033 bacterial contigs from the *Peltigera membranacea* metagenome yielded mupltiple hits on several genes involved in lichen secondary metabolite resistance, inorganic phosphate mobilization, biopolymer degradation and several other potentially important functions in thallus colonization and symbiosis, as well as several genes likely to play a role in plant pathogenicity. Among plant virulence genes found in the metagenome are several homologs of the virB/D4 type IV secretion system from diverse bacteria (xanthomonads, burkholderiae, sphingomonads, and acidobacters).
Phenotypic analysis of selected isolates indicated that biopolymer hydrolytic activity is common among these bacteria, as is inorganic phosphate mobilization and nitrogen fixation. Several of the isolated pseudomonads are very efficient producers of surfactants and many of them are capable of twitching motility. Antagonism against the plant pathogenic fungus *Pythium myriotylum* has been observed for several isolates.

Key Words: Lichen-associated bacteria, Endothallic bacteria, Symbiosis, Environmental reservoirs, Metagenomics.

REFERENCES

SIGURBJÖRNSDÓTTIR, M. A.; ANDRÉSSON, Ó. S.; VILHELMSSON, O. (2015): Analysis of the *Peltigera membranacea* metagenome indicates that lichen-associated bacteria are involved in phosphate solubilization. Published ahead of print in Microbiology, March 3, 2015. DOI: 10.1099/mic.0.000069.
GRUBE, M.; BERG, G.; ANDRÉSSON, Ó. S.; VILHELMSSON, O.; DYER, P. S.; MIAO, V. (2013): Lichen genomics: prospects and progress. In MARTIN, F. (ed.) Ecological Genomics of Fungi, 191-212. Wiley-Blackwell, Oxford, UK. ISBN: 978-1-119-94610-6.

P19. A low frequency persistent reservoir of a genomic island in a pathogen population ensures island survival and improves pathogen fitness in a susceptible host.

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The co-evolution of bacterial plant pathogens and their hosts is a complex and dynamic process. *Pseudomonas syringae* pv. *phaseolicola* (*Pph*) is the causative agent of halo blight in the common bean *Phaseolus vulgaris*. Gene-for-gene interactions underpin va-rietal resistance and race structure in the Pph/bean interaction. Pph race 4 strain 1302A contains the effector gene *avrPphB* (*hopAR1*), which matches the resistance gene R3 and causes a rapid resistant reaction called the hypersensitive response (HR) in bean cultivar Tendergreen. The antimicrobial environment generated within the plant causes excision of PPHGI-1 and its loss from the genome. Cells that lack PPHGI-1 are able to proliferate and cause disease in the plant (Pitman et al., 2005). The evolution of virulent strains of 1302A occurs in Tendergreen due to the selection pressure on the pathogen to bypass the HR caused by *avrPphB*, however, we have never observed 100% loss of PPHGI-1 from the *Pph* population (Lovell et al., 2011). We have developed a mathematical model to predict if the genomic island PPHGI-1 would be maintained in the population long term. We then empirically tested the predictions made by the model and determined that PPHGI-1 frequency in the bacterial population drops during the HR until the HR is no longer triggered. The island is then stably maintained in approximately 0.5% of the population over the long term. However, when a population of *Pph* that contains 0.5% cells carrying PPHGI-1 is inoculated into a bean cultivar that does not produce the HR, the proportion carrying PPHGI-1 increases rapidly suggesting that PPHGI-1 confers a fitness benefit. These results provide insights into the long term population dynamics of genomic island retention and loss.

Key Words: *Pseudomonas, syringae* pv. *phaseolicola*, bean, genomic island.

REFERENCES

Lovell, H.C., Jackson, R.W., Mansfield, J.W., Godfrey, S.A., Hancock, J.T., Desikan, R., Arnold, D.L., 2011. In planta conditions induce genomic changes in *Pseudomonas syringae* pv. *phaseolicola*. Mol. Plant Pathol. 12, 167–176.
Pitman, A.R., Jackson, R.W., Mansfield, J.W., Kaitell, V., Thwaites, R., Arnold, D.L., 2005. Exposure to host resistance mechanisms drives evolution of bacterial virulence in plants. Curr. Biol. 15, 2230–2235.

P20. Quantum pathogen evolution by integron-mediated effector capture.

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Plant pathogenic Pseudomonads are responsible for the loss of millions of pounds in crop revenue each year. Pseudomonads export effector molecules into the plants' cells in order to supress immune responses such as the plant hypersensitive response (HR). The plants immune system can recognise certain effector molecules and trigger the HR preventing bacterial infection. Pseudomonads can evade HR by potentially gaining different effector molecules captured within integrons, mobile pieces of DNA that have the ability to capture and express genes from different pathovars and species. It is not known how widespread integrons are amongst Pseudomonads although there are genes conserved in integrons that can be identified such as *xerC*/*D*. The gene *rulB* appears to be a hotspot for integron insertion (Rhodes et al., 2013). An integron-like element was previously identified in *Pseudomonas syringae* pv. *pisi* strain 203 which is inserted into *rulB* (Arnold et al., 2000). In this study the abundance of integron-like elements were screened for in 141 *Pseudomonas syringae* strains and 23 other *Pseudomonas* species. The three regions screened were the *rulAB* gene, the *xerC*/*D* gene and the integron insertion junction region, *rulB*-*xerC*/*D*. The screening found that 16.5% of the strains contained an intact *rulAB* gene, 34.8% of the strains contained a *xerC*/*D* gene and 28% contained a disrupted *rulB* indicative of integron insertion. The mobility of any identified integron-like elements was also assessed using conjugation matings to see if the integron-like element could move from the chromosome into *rulB* on the plasmid pWW0. Future work will include examining the conditions required for integrons to capture effector genes, investigating the integrons' colocalisation with *rulAB*, determining whether an integron will capture a novel effector to overcome plant resistance and testing if the disruption to *rulAB* causes an adverse effect on UV damage repair function.

Key Words: *Pseudomonas*, integron, *rulAB*, *xerC*, integron-like element.

REFERENCES

Rhodes, G, Bosma, H, Studholme, D, Arnold, D.L, Jackson, R.W. and Pickup, R.W. (2013). The *rulB* gene of plasmid pWW0 is a hotspot for the site-specific insertion of integron-like elements found in the chromosomes of environmental *Pseudomonas fluorescens* group bacteria. Environmental Microbiology. 16(8): pp.2374-2388.
Arnold, D.L, Jackson, R.W. and Vivian, A. (2000). Evidence for the mobility of an avirulence gene, *avrPpiA1*, between the chromosome and plasmids of races of *Pseudomonas syringae* pv. *pisi*. Molecular Plant Pathology. 1(3): pp195-199.

P21. Characterizing the regulon of the two-component system, PSPTO_3380 and PSPTO_3381

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The two-component system, PSPTO_3380 (3380) and PSPTO_3381 (3381) in *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) is involved in pathogenicity. We have reported that addition of a number of divalent cations to the medium induces expression of this two-component system. 3380/3381 regulates transcription of itself and of several genes including PSPTO_5255 (5255), which encodes for a carbonic anhydrase. To gain further insight into the role of this two-component system, we compared the region upstream of the open reading frame that encodes 3380/3381 to the orthologous regions in all sequenced Pseudomonads. Then we used the motif finder MEME to search for a binding motif for 3381. The putative binding motif generated consists of two direct repeats separated by six random base pairs. This is characteristic of OmpR-family response regulators, of which 3381 is a member. By generating a Hidden Markov model we were able to find 17 putative binding sites in the *Pst* genome. There are several 3381 binding sites within intergenic regions followed by a Rho-independent terminator, one of which is upstream of 5255. This suggests that 3381 regulates the transcription of small RNAs at these sites. Using quantitative reverse transcriptase PCR we verified transcriptional activity in a subset of these regions. When comparing RNA isolated from wild-type and Δ3380 or Δ3381 strains we saw differential gene expression for some of these areas, including the region upstream of 5255. The discovery of PSPTO_3380/3381 –regulated small RNAs adds complexity to the regulon of this two-component system and allows for the possibility of indirect regulation of a large number of genes. Further characterization of these small RNAs will help define the means by which 3380/3381 regulates virulence of *Pst*.

Key Words: signal transduction, two-component system, small RNAs, gene regulation, transcriptome.

P22. The T3SS effector repertoire of *Pseudomonas savastanoi* pv. *savastanoi* DAPP-PG722 and NCPPB3335 only differ in the putative effector HOPA1'

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The virulence of *Pseudomonas savastanoi* pv. *savastanoi* (*Psv*), which causes hyperplastic symptoms (knots) on olive plants, has been mainly related to the phytohormones indole-3-acetic acid and cytokinins produced by the bacterium and the effectors (T3Es) injected in the host cells through the type III secretion system (T3SS). In addition, the virulence is regulated by the quorum sensing intercellular communication system mediated by N-acyl homoserine lactones (Hosni et al., 2011), as well as by a collection of other factors recently identified (Matas et al., 2012).
Since *Psv* NCPPB3335, isolated in France, is more virulent than the Italian strain *Psv* DAPP-PG722, both in adult and in micropropagated olive plants, we performed a comparative analysis on their genomes in order to understand the molecular basis of the different virulence degree.
Bioinformatics analysis and PCR amplifications revealed that the two strains share all 33 T3Es reported for NCPPB3335 (Matas et al., 2014), while the *hopA1'* gene was only present in the DAPP-PG722 genome. BLASTp searches revealed that *HopA1'* is not present in all strains of the *P. syringae* complex and its amino acidic sequence has a high similarity (92%) with that of HopA2 from *P. syringae* pv. *aesculi* strain 2250. Dot-blot analysis and southern blot hybridizations, performed on 31 *Psv* strains isolated in different countries and on *P. syringae* strains isolated from either woody or herbaceous hosts, demonstrated that *hopA1'* sequences are only detected in bacteria of the *P. syringae* complex infecting woody hosts.
To investigate the role of HopA1' in the virulence of *Psv*, its ectopic expression in *Psv* NCPPB3335 was performed and inoculation in micropropagated and adult olive plants are in progress.

Key words: Genome comparative analysis, virulence, putative effector

REFERENCES

HOSNI, T.; MORETTI, C.; DEVESCOVI, G.; SUAREZ-MORENO, Z.R.; FATMI, M.; GUARNACCIA, C.; PONGOR, S.; ONOFRI, A.; BUONAURIO, R.; VENTURI, V.; (2011): Sharing of quorum-sensing signals and role of interspecies communities in a bacterial plant disease. The ISME Journal, 5, 1857.
MATAS, I.M.; LAMBERTSEN, L.; MORENO, L.R; RAMOS, C. (2012): Identification of novel virulence genes and metabolic pathways required for full fitness of *Pseudomonas savastanoi* pv. *savastanoi* in olive (olea europea) knots. New Phytol, 196(4): 1182-96.
MATAS, I.M.; CASTAŃEDA-OJEDA, M.P.; ARAGON, I.M.; ANTUNEZ-LAMAS, M.; MURILLO, J.; RODRIGUEZ-PALENZUELA, P.; LOPEZ-SOLANILLA, E.; RAMOS, C. (2014): Translocation and functional analysis of *Pseudomonas savastanoi* pv. *savastanoi* NCPPB 3335 Type III Secretion System Effectors reveals two novel effector families of the Pseudomonas syringae complex. MPMI 27 (5), 424–436.

P23. HCP secretion island (HSI)-II encodes the only type VI secretion system for inter-bacterial competition in *Pseudomonas syringae* pv. *tomato* DC3000

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The phytopathogenic bacterium *Pseudomonas syringe*, which can infect a wide array of plants, has been used to investigate plant-microbe interaction and pathogenesis for decades. In the model phytopathogenic bacterium *P. s.* pv. *tomato* DC3000 (*Pst* DC3000), two gene clusters, namely Hcp secretion island (HSI)-I and HSI-II, were identified and may code for type VI secretion systems (T6SSs). In this study, the regulation and functions of these two gene clusters in Pst DC3000 were characterized in details. We found that HSI-I did not express in either King's B or *hrp*-inducing media, and HSI-II contains three operons. By means of a GUS reporter system under the control of different the upstream se-quence of each operon in HSI-I and HSI-II clusters, the highest expression can be detected for the second operon in HSI-II cluster, and each operon responds to different sugars in a different manner. Systemic deletion of each gene in the HSI-II cluster revealed that several genes are indispensable for the secretion function of type VI secretion system. In addition, the competition assay showed that many genes in HSI-II cluster are required for both contact-dependent interbacterial competition and in planta growth of *Pst* DC3000. Alt-hough two putative gene clusters for T6SS were discovered in *Pst* DC3000, as our data indicated, the HSI-II cluster is the only one functions in growth fitness. However, the mechanisms underlying the HSI-II-mediated competition ability needs further investigation.

Key Words*: Pseudomonas syringae*, T6SS, and Hcp2

P24. Characterization of PSPTO_0281 gene in phytopathogenic *Pseudomonas syringae* pv. *tomato* DC3000

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Pseudomonas syringae is a rod-shaped, Gram-negative bacterium with polar flagella. As a plant pathogen with broad host range and economical importance, *P. syringae* has become one of the most studied model phytopathogen. From previous studies, we have known that type III secretion system (T3SS) plays a crucial role in the pathogenicity of *P. syringae*. In addition, the type VI secretion system (T6SS) has been shown tobe involved in the fitness of interbacterial competition. By means of RNA sequencing to identify gene differentially expressed in *P. s.* pv. *tomato* DC3000 wild type and Δ*sfa2*, we discovered one gene, PSPTO_0281, which was highly expressed in Δ*sfa2*. Although PSPTO_0281 was predicted to encode a putative histone-like nucleoid structuring (H-NS) protein, whether it acts as a transcriptional regulator remains to be elucidate. Using qRT-PCR, GUS activity and Western blot analysis, the expression and secretion of Hcp2 were found independent of PSPTO_0281. However, overexpression of PSPTO_0281 slightly decreased the interbacterial competition ability of *Pst* DC3000 against *E. coli* MG1655 and *P. s.* pv. *phaseolicola* 1448a. Considering the reciprocal regulation of T6SS and Type III secretion system (TTSS) in the gene regulatory network, TTSS-related genes were further analyzed. Indeed, PSPTO_0281 as well as Sfa2 both regulate the expression of TTSS regulators and effector, including *hrpR*, *hrpL* and *avrPto*. In the pathogenicity assay, over-expression of PSPTO_0281 enhanced virulence in Pst DC3000 as shown in Δ*sfa2* mutant. Lastly, we also verified that PSPTO_0281 encode a DNA-binding protein, which bind to DNA nonspecifically.

Key Words: *Pseudomonas syringae*, type III secretion system (T3SS), type VI secretion system (T6SS), histone-like nucleoid structuring (H-NS) protein.

REFERENCES

Mougous, J.D.; M.E. Cuff. (2006): A virulence locus of *Pseudomonas aeruginosa* encodes a protein secretion apparatus. Science. 312:1526-1530.
Eijkelkamp, B.A., U.H. Stroeher (2013): H-NS plays a role in expression of *Acinetobacter baumannii* virulence features. Infection and immunity. 81:2574-2583.

P25. MRM-MS quantitative proteomics applied to the *Pseudomonas syringae* secretome

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The plant pathogen *Pseudomonas syringae* pv. *tomato* (DC3000) causes virulence by delivering effector proteins into host plant cells through its type three secretion system (T3SS). In response to the plant environment DC3000 expresses hypersensitive response and pathogenicity genes (*hrp*). Pathogenesis depends on the ability of the pathogen to manipulate the plant metabolism and to inhibit plant immunity, which depends to a large degree on the plant's capacity to recognise both pathogen and microbial determinants (PAMP/MAMP-triggered immunity). We have developed and employed MS-based shotgun and targeted proteomics to (i) elucidate the extracellular and secretome composition of DC3000 and (ii) evaluate temporal features of the assembly of the T3SS and the secretion process together with its dependence of pH. The proteomic screen, under *hrp* inducing *in vitro* conditions, of extracellular and cytoplasmatic fractions indicated the segregated presence of not only T3SS implicated proteins such as HopP1, HrpK1, HrpA1 and AvrPto1, but also of proteins not usually associated with the T3SS or with pathogenicity. Using multiple reaction monitoring MS (MRM-MS) to quantify HrpA1 and AvrPto1, we found that HrpA1 is rapidly expressed, at a strict pH-dependent rate and is post-translationally processed extracellularly. These features appear to not interfere with rapid AvrPto1 expression and secretion but may suggest some temporal post-translational regulatory mechanism of the T3SS assembly. The high specificity and sensitivity of the MRM-MS approach should provide a powerful tool to measure secretion and translocation in infected tissues (1).

Key Words: *Pseudomonas syringae*, MRM-MS, proteomics, secretome, HrpA

REFERENCES

1. Schumacher, J., Waite, C. J., Bennett, M. H., Perez, M. F., Shethi, K., and Buck, M. (2014) Differential secretome analysis of *Pseudomonas syringae* pv *tomato* using gel-free ms proteomics, Frontiers in Plant Science 5, 242.

P26. PSPTO_5415 and PSPTO_5436 encode two major *vgrG* homologs involved in interbacterial competition ability of *Pseudomonas syringae* pv. *tomato* DC3000

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The type VI secretion system (T6SS), found in most Gram-negative bacteria, is a macromolecular structure analogous to bacteriophage tails, with Hcp stacking to form a tail-tube like structure and VgrG acting as a puncturing device at the tip of this tube. The plant pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 is known to have two putative T6SS gene clusters, namely HSI-I and HSI-II. Hcp2, encoded in the HSI-II gene cluster, was previously shown to be secreted and play an essential role in antimicrobial activity against enterobacteriaceae. However, the role of VgrG in this bacterium is still unknown. A total of seven *vgrG* genes were predicted in *Pst* DC3000, three of which are located within the HSI-I and HSI-II gene clusters with additional four scattered in the bacterial genome. Here, we demonstrate the secretion of VgrG proteins, and also, the contribution of each VgrG protein in secretion and interbacterial competition by systematic deletion of these genes. Among these VgrG homologs, VgrG-2b (PSPTO_5436) was the major contributor in secretion and interbacterial competition activity against *E. coli* and five different phytopathogenic bacteria followed by a small contribution of VgrG-2a (PSPTO_5415). Both VgrG proteins are encoded within the HSI-II gene cluster, and this also support the notion that HSI-II plays a major role in *Pst* DC3000 to compete for niches, thus conferring this bacterium significant fitness advantage against competitive microbes. Additionally, eleven putative VgrG-associated genes were targeted for deletion to observe its role in the T6SS, and the results showed a potential effector activity for PSPTO_5413, which is located downstream of VgrG-2a.

Key Words: Type VI secretion system, VgrG, interbacterial competition.

REFERENCES

HAAPALAINEN, M.; MOSORIN, H.; DORATI, F.; WU, R.-F.; ROINE, E.; TAIRA, S.; NISSINEN, R.; MATTINEN, L.; JACKSON, R.; PIRHONEN, M.; LIN, N.-C. (2012). Hcp2, a Secreted Protein of the Phytopathogen *Pseudomonas syringae* pv. *tomato* DC3000, is Required for Fitness for Competition against Bacteria and Yeasts. The Journal of Bacteriology, 194 (18), 4810-4822.

P27. The expression of *Pseudomonas syringae* type III secretion system presents phenotypic variation *in planta*

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Genetic diversity is essential for the process of natural selection. Individuals with particular genotypes that enable them to outgrow competitors, survive stresses, and adapt to different environments, will be selected. Whereas sexual reproduction and meiotic recombination are the main sources of genetic variation in eukaryotes, mutation, recombination and horizontal transfer have been traditionally considered the sources of variation in prokaryotes. However, the development of single-cell analyses such as flow cytometry, microfluidics, or fluorescent microscopy, has challenged the notion of the bacterial population as a clonal, and therefore phenotypically homogeneous. The phenotypic variation described within clonal populations of many bacterial human and animal pathogens, affecting virulence traits, and including aspects of clinical relevance. However, little is known about the impact of phenotypic variation on plant pathogenic bacteria.
In this work, we apply single-cell analysis to monitor the expression of genes encoding two key structural and regulatory elements of the type III secretion system, and the translocated effector HopAB1 of the plant pathogenic bacteria *Pseudomonas syringae*, and demonstrate they display heterogeneous expression levels within the host plant. Further, we apply fluorescent confocal microscopy to illustrate how bistability of bacterial virulence factors can affect host adaption, by analysing the development within the host of mixed *P. syringae* populations with key differences in these genes. We analyse the cellular basis that determine whether co-existing *P. syringae* bacteria that differ in their expression of these key virulence factors grow different that they would in a clonal infection, and discuss the potential implications for pathogen evolution.

Key Words: phenotypic variation, type III secretion system, host adaptation, confocal microscopy, flow cytometry.

P28. Systematic characterization of a novel citrus pathogenic species of the *Pseudomonas syringae* phylogenetic group

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In a screening of plant pathogenic bacteria, five citrus pathogenic strains, designated FBF24, FBF58, FBF102, FBF103 and FBF122, were isolated from citrus orchards in Northern Iran with symptoms of disease in leaves and stems. The strains were analyzed by a taxonomic polyphasic approach. A Multilocus sequence analysis (MLSA) based on the 16S rRNA, *gyrB* and *rpoD* gene sequences clustered the strains in a single branch in the *Pseudomonas syringae* group and revealed *Pseudomonas meliae*, *Pseudomonas caricapapayae* and *Pseudomonas tremae* as the closest relatives with 91.6-91.7% of similarity. DNA-DNA hybridizations showed DNA-DNA relatedness values with the type strain of *P. syringae* and with other species type strains of the *P. syringae* group lower than 25%, supporting the classification of the five strains within a novel species of the genus *Pseudomonas*. The strains were characterized phenotypicaly and clustered in the same phenon in a numerical taxonomy study. The strains were Gram-negative, strictly aerobic, rod-shaped and motile, fluorescent on King B plates, oxidase negative, aesculin positive and negative for urea and gelatin tests. None strain produced levan and were negative for potato root test. Only the strain FBF102 produced a hypersensitivity reaction on tobacco plants.
The genome of the type strain proposed (FBF102T) was sequenced by Illumina technology to determine the phylogenomic position of the novel species and also to detect the genes involved in the pathogenic characteristics.
For this new species, the name *Pseudomonas caspian* sp. nov. is proposed, and the type strain is FBF102T.

Key words: *Pseudomonas*, Taxonomy, Citrus, Genome, Pathogenicity

P29. Rapid identification of bacteria associated with acute oak decline using HRM analysis

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Acute oak decline (AOD) is serious and increasing problem in Britain, affecting the native oak species *Quercus robur* and *Q. petraea* throughout the Midlands and south to south-east of the country. Symptoms include stem bleeding from vertical cracks between bark plates, stained tissues underlying the bleeding patches and necrosis leading to the for-mation of cavities in the inner bark. Larval galleries of the bark boring buprestid *Agrilus biguttatus* are frequently found in close proximity to necrotic tissue. Numerous bacterial strains from several different families have been isolated from necrotic lesions, fluid exudates and occasionally larval galleries of symptomatic trees. The causal agent has yet to be determined, but it appears that two newly-described bacterial species, *Gibbsiella quercinecans* and *Brenneria goodwinii* (Brady et al., 2010, Denman et al., 2012), may be responsible for the symptoms. With an increasing number of woodlands and parklands affected by AOD, new isolations from infected tissue are performed on a regular basis, highlighting the need for a single, rapid method for the improved identification and detection of both *G. quercinecans* and *B. goodwinii*. A novel technique, High Resolution Melt (HRM) analysis targeting a short region of the *atpD* gene containing single nucleotide polymorphisms (SNPs), was applied to the most frequently isolated bacteria from symptomatic oak. Following amplification in the presence of an intercalating dye, which binds specifically to double-stranded DNA, samples were subjected to a melting step from 50 to 90 °C. The level of fluorescence for each amplified strain was plotted against temperature and a melt curve was generated. A unique HRM profile was produced for each species due to the SNPs between species, allowing rapid identification and differentiation of *G. quercinecans* and *B. goodwinii* in a single assay. This method greatly reduces the time taken to identify the bacteria present in oak tissue samples.

Key Words: *Gibbsiella*, *Brenneria*, AOD, oak, identification

REFERENCES

BRADY, C.L., DENMAN, S., KIRK, S., VENTER, S.N., RODRÍGUEZ-PALENZUELA, P., COUTINHO, T.A. (2010): Description of *Gibbsiella quercinecans* gen. nov., sp. nov, associated with Acute Oak Decline. Systematic and Applied Microbiology, 33, 444-450.
DENMAN, S., BRADY, C.L., KIRK, S., CLEENWERCK, I., VENTER, S.N., COUTINHO, T.A., DE VOS, P. (2012): *Brenneria goodwinii* sp. nov., associated with acute oak decline in the UK. International Journal of Systematic and Evolutionary Microbiology, 62, 2451-2456.

P30. The kiwifruit phyllosphere – a playground for *P. syringae*

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Epidemics caused by emerging plant diseases have a tremendous effect on primary food production. The plant pathogen *Pseudomonas syringae* pv. *actinidiae* (*Psa*) is the causative agent of bacterial canker on kiwifruit (*Actinidia* spp.) and was first described on green kiwifruit in 1984 [1]. In 2008 a very virulent and aggressive form of *Psa* was reported for the first time on golden kiwifruit in Italy and was followed by a global pandemic [2].
Bacteria densely populate the phyllosphere (10^6-10^7 cfu cm^{-2}) [3] and various commensal and/or mutualistic members of the *P. syringae* complex can be present. Novel genomic content that is horizontally transmitted between closely related strains could result in the emergence of a new virulent form of *Psa*. Hence it is important to identify potential donor strains from the natural environment in commercial orchards, as these bacteria can be regarded as a source population of new genetic material.
The aim of my study is to gain an understanding of the genetic diversity and phylogenetic relations of *Pseudomonas syringae* occupying the leaf surface in *Actinidiae* sp. (kiwifruit) during the current outbreak of *Psa*.
During the growing season of 2013/2014 we collected bacterial isolates from *Psa* infected and non-infected orchards from two cultivars. Multi Locus Sequence Typing (MLST) of four housekeeping genes was done for a total of 140 *P. syringae* isolates, as well as 90 *Pseudomonas* sp. isolates.
Preliminary data suggests that the collection of strains encompasses various representatives of four major monophyletic groups of *Pseudomonas syringae*. Curiously we have recovered a clade of pv. *phaseolicola* isolates in NZ, which has similarly been shown for a MLST analysis of a collection of strains isolated in China. Further analysis will help to understand the effect of microevolutionary processes on patterns of *Psa* diversity.

Key Words: Psa, MLST, population structure, phyllosphere, diversity

REFERENCES

[1] SERIZAWA, S.; ICHIKAWA, T.; TAKIKAWA, Y.; TSUYUMU, S.; Goto M (1989) Occurrence of bacterial canker of kiwifruit in Japan: description of symptoms, isolation of the pathogen and screening of bacteriocides. Annals of the Phytopatholgoical Society of Japan, 55, 427-436.
[2] BALESTRA, G. M.; MAZZAGLIA, A.; QUATTRUCCI, A.; RENZI, M.; ROSSETTI, A. (2008) Occurrence of *Pseudomonas syringae* pv. *actinidiae* in Jin Tao kiwi plants in Italy. Phytopathologia Mediterranea, 48, 299-301.
[3] BULGARELLI, D.; SCHLAEPPI, K.; SPAEPEN, S.; VER LOREN VAN THEMAAT, E.; SCHULZE-LEFERT, P. (2013) Structure and Functions of the Bacterial Microbiota of Plants. Annual Reviews of Plant Biology, 64, 807-838.

P31. Molecular characterization of *Pseudomonas syringae* from stone fruits in Lebanon using rep-PCR

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In order to evaluate the sanitary status of stone fruits in Lebanon regarding bacterial diseases, a total of 136 *Pseudomonas syringae* were isolated from 303 samples collected during a survey in spring 2013. Those isolates were identified according to morphological, physiological and biochemical tests which divided them between the two pathovars *syringae* (*Pss*) and *morsprunorum* race 1 (*Psm1*). In fact none of the other bacteria pathogenic to stone fruits was isolated during this survey reporting that bacterial canker caused by those two pathovars is the main bacterial disease of stone fruits in Lebanon.
Lately, the collected isolates were assessed by rep-PCR using BOX primer, and for the presence of a specific Hop protein coded by Psyr_1890 gene, not present on other *P. syringae* pathovars. The unweighted pair-group method using arithmethic averages analysis (UPGMA) of genomic fingerprints revealed 20 different patterns which can be divided into three major groups (A, B and C). Group A assemble all Psm1 isolates (29) and one isolate supposed to be Pss according to biochemical tests. Group B assemble 17 Pss isolates where 10 of them were negative when tested for the presence of Psyr_1890 gene. Finally, group C assemble the majority of the Lebanese isolates (89) where most of them belong to the patterns 1, 2 or 4. All of them were positive when tested for Psyr_1890 gene with the exception of one isolate. No clear distinction was observed between host of isolation and phylogenetic classification with the exception of the Pss isolated from apricot that were almost homogenous belonging all to group C.

Key words: Survey, *Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *morsprunorum*, rep-PCR, Phylogenetic classification.

REFERENCES

VERSALOVIC, J. ; SCHNEIDER, M. ; DE BRUIJN, F. J. ; LUPSKI, J. R. (1994). Genomic Fingerprinting of Bacteria Using Repetitive Sequence Based PCR (Rep-PCR). Methods in molecular and cellular biology, 5, 25-40.
VIEIRA, J.; MENDES, M. V. ; ALBUQUERQUE, P.; MORADAS-FERREIRA, P.; TAVARES, F. (2007). A novel approach for the identification of bacterial taxa-specific molecular markers. Letters in Applied Microbiology, 44 (5), 506-512.

P32. Identification, subcellular localization and in planta recognition of pathogenicity effectors of *Plasmopara halstedii*, the oomycete responsible for downy mildew in sunflower, *Helianthus annuus*

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Plasmopara halstedii is an obligate biotroph oomycete causing downy mildew disease on sunflower, an economically important cultivated crop. Disease symptoms observed in fields, plant dwarfism, leaf bleaching, sporulation and production of infertile flowers, impair strongly seed yield. *P. halstedii* pathotypes are defined by their divergent virulence profiles in a set of sunflower differential hosts carrying different Pl Resistance genes, not yet cloned. Number of pathotypes increased to 16 during the last 25 years in France, concomitantly with the breakdown of Pl resistance loci used in fields. Studying disease infec-tion mechanisms and pathogen molecular determinants is a prerequisite for deciphering plant sustainable resistance.
In oomycetes, two classes of effectors are translocated into the host plant: RXLRs and CRNs. Sequencing of cDNA from *P. halstedii* spores and sunflower infected tissues was complemented with Illumina genomic sequencing of 7 *P.halstedii* pathotypes leading to identification of 250 putative RXLR and CRN expressed effector genes. The polymor-phism of a subgroup of 54 effectors was analyzed in *P. halstedii* pathotypes. Compared to 125 *P. halstedii* non-effector genes, effector genes showed a higher proportion of non synonymous mutations, suggesting that effector genes were not subjected to the same evolutionary forces. Subcellular localization of selected candidate effectors fused to GFP is currently being done by transient expression experiments in sunflower leaf cells. Triggering of defense reactions (HR) in resistant versus susceptible near isogenic sunflower lines should lead to identification of *P.halstedii* avirulence genes.

Key Words: effector polymorphism; *Plasmopara halstedii*; *Helianthus annuus*; pathogen virulence evolution; obligate biotroph oomycete.

REFERENCES

GASCUEL, Q., MARTINEZ, Y., BONIFACE, M.-C., VEAR, F., PICHON, M., GODIARD, L. (2015) The sunflower downy mildew pathogen *Plasmopara halstedii*. Mol. Plant Pathol. 16, 109-122.
AS-SADI, F., CARRERE, S., GASCUEL, Q., HOURLIER, T., RENGEL, D., LE PASLIER, M.-C., BORDAT, A., BONIFACE, M.C., BRUNEL, D., GOUZY, J., GODIARD, L. AND VINCOURT, P. (2011) Transcriptomic analysis of the interaction between *Helianthus annuus* and its obligate parasite *Plasmopara halstedii* shows single nucleotide polymorphisms in CRN sequences. BMC Genomics 12, 498-513.

P33. Genomic plasticity enables phenotypic variation of *Pseudomonas syringae* pv. *tomato* DC3000

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Whole genome sequencing revealed the presence of a genomic anomaly in the region of 4.7 to 4.9 Mb of the *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 genome. The average read depth coverage of *Pst* DC3000 whole genome sequencing results suggested that a 165 kb segment of the chromosome had doubled in copy number. Further analysis confirmed the 165 kb duplication and that the two copies were arranged as a direct tandem repeat. Examination of the corresponding locus in *Pst* NCPPB1106, the parent strain of *Pst* DC3000, suggested that the 165 kb duplication most likely formed after the two strains diverged via transposition of an ISPsy5 insertion sequence (IS) followed by unequal crossing over between ISPsy5 elements at each end of the duplicated region. Deletion of one copy of the 165 kb region demonstrated that the duplication facilitated enhanced growth in some culture conditions, but did not affect pathogenic growth in host tomato plants. These types of chromosomal structures are predicted to be unstable and we have observed resolution of the 165 kb duplication to single copy and its subsequent re-duplication. These data demonstrate the role of IS elements in recombination events that facilitate genomic reorganization in *P. syringae* and that these events can influence the phenotype of the bacteria.

Key Words: *Pseudomonas syringae* pv. *tomato* DC3000, Recombination, Genome Rearrangements, Genomic Duplication, Transposition.

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