Conference Overview

Registration: Tuesday 5:00 – 7:00 pm (IBIS) / Wednesday 7:30 – 8:30 am (IBIS)

Wednesday 13rd December	Thursday 14th December	Friday 15th December
9:15 – 9:30 Welcome		
9:30 – 11:00 WG 1 Diagnostics & Diversity – Population Structure	**8:30 – 10:30** Social Program	**9:15 – 10:45** WG 4 Disease Management – Vector Control
Break	**Break**	**Break**
11:30 – 12:50 WG 1 Diagnostics & Diversity – Population Structure	**11:00 – 12:30** WG 3 Genetic Resistance – Host Defence	**11:15 – 12:15** WG 4 Disease Management – Vector Control
12:50 – 14:20 Lunch	**12:30 – 14:00** Lunch	**12:15 – 12:25** Wrap-up WG4
14:20 – 15:00 WG 1 Diagnostics & Diversity – Population Structure	**14:00 – 15:20** WG 3 Genetic Resistance – Host Defence	**12:25 – 12:45** Concluding remarks by the organisers
15:00 – 15:10 Wrap-up WG1	**15:20 – 15:30** Wrap-up WG3	**12:45 – 14:15** Lunch
15:10 – 16:00 WG 2 Pathogen Biology	**15:30 – 16:00** Presentation of Activities of the EuroXanth COST Action & Plenary Discussion	
Break	**Break**	
16:30 – 17:50 WG 2 Pathogen Biology	**16:30 – 18:00** Poster Session	
17:50 – 18:00 Wrap-up WG2	**16:30 – 18:00** Management Committee Meeting	
	19:30 Walk by the Old City to the Gala Dinner	

1st Annual Conference of the EuroXanth COST Action

Integrating Science on *Xanthomonadaceae* for integrated plant disease management in Europe

Organisers

Joana Costa	FitoLab – Instituto Pedro Nunes and University of Coimbra, Portugal
Ralf Koebnik	IRD, Montpellier, France

Scientific Committee

Jens Boch	Leibniz University Hannover, Germany
Vittoria Catara	University of Catania, Italy
Joana Costa	University of Coimbra, Portugal
Maria Leonor Cruz	Instituto Nacional de Investigacao Agraria e Veterinaria, Oeiras, Portugal
Ralf Koebnik	IRD, Montpellier, France
Tamas Kovacs	ENVIROINVEST, Hungary
Joël F. Pothier	Zürich University, Switzerland
Nicholas Skandalis	Benaki Phytopathological Institute, Greece
Emilio Stefani	Università degli Studi di Modena e Reggio Emilia, Italy
Fernando Tavares	Universidade do Porto, Portugal

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Scientific Program

Wednesday 13th of December

9:15-9:30 **Opening & welcome by the organisers**
Joana COSTA *(Organiser)* & **Ralf KOEBNIK** *(Chair)*

Session 1 Diagnostics & Diversity – Population Structure

Chairs: Joana COSTA & Nicholas SKANDALIS

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9:30-10:00 – **Marie-Agnès JACQUES** ***(INRA, FR):*** *Is* Xylella fastidiosa *really emerging in France?*

10:00-10:20 – **Vittoria CATARA** ***(University of Catania, IT)*****:** *Identification and distribution of prevalent races of* Xanthomonas campestris *pv.* campestris *in Italy*

10:20-10:40 – **Camila FERNANDES** ***(University of Porto, PT):*** Xanthomonas arboricola *pv.* juglandis*: an endemic walnut pathogen in Portugal*

10:30-11:00 – **Christian VERNIÈRE** ***(CIRAD, FR****): Molecular epidemiology of* Xanthomonas euvesicatoria *strains from the Balkan Peninsula revealed by a new multiple-locus variable-number tandem-repeat analysis scheme*

11:00-11:30 – COFFEE BREAK

11:30-11:50– **Jaime CUBERO** ***(INIA, ES)*****:** *Genomic-based development of a real-time PCR protocol for improving the diagnosis of* Xanthomonas arboricola *pv.* pruni *the causal agent of bacterial spot disease of Prunus spp.*

11:50-12:10– **Ioannis THEOLOGIDIS** ***(Institute of Molecular Biology and Biotechnology, GR):*** *Development of new diagnostic tools based on the type III secretion system of Gram- phytopathogenic bacteria*

12:10-12:30– **Fernando TAVARES** ***(University of Porto, PT):*** *Guidelines to discover new xanthomonads species-specific markers for detection and genotyping: from the in silico analysis to experimental validation*

12:30-12:50 – **Christophe SOLA** ***(Paris Saclay, FR):*** *Postgenomic lateral transfer knowledge from* Salmonella enterica*,* Mycobacterium tuberculosis*,* Legionella pneumophila *CRISPRs genetic diversity knowledge and expertise to a new model:* Xanthomonas oryzae

12:50-14:20 – LUNCH

14:20-14:40 – **Monika KAŁUŻNA** ***(Research Institute of Horticulture, PL)****: Present situation and study on* Xanthomonas arboricola *in Poland*

14:40-15:00 – **Joana VICENTE** ***(School of Life Sciences, UK):*** *New species of* Xanthomonas *from watercress*

15:00-15:10 – **Wrap-up of Session 1**

Session 2 Pathogen Biology

Chairs: Joël F. POTHIER & Guido SESSA

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15:10-15:40 – **Matthieu ARLAT** ***(INRA, FR):*** *Comparative genomics of the order* Xanthomonadales*: a glimpse of pathogenicity evolution of* Xanthomonas *and* Xylella *genera*

15:40-16:00 – **Stefanie MÜCKE** ***(Leibniz Universität Hannover, DE)****:* Xanthomonas *redirects host defense metabolism to benefit infection*

16:00-16:30 – COFFEE BREAK

16:30-16:50 – **Joanna PULAWSKA** ***(Research Institute of Horticulture, PL):*** *Transcriptome analysis of* Xanthomonas fragariae *in strawberry plants with application of RNA-seq*

16:50-17:10 – **Alice BOULANGER** ***(INRA, FR):*** *Genetics of bacterial fitness of* X. campestris *in vitro and in planta*

17:10-17:30 – **Suayib ÜSTÜN** ***(Swedish University of Agricultural Sciences, SW)****: How* Xanthomonas *hijacks the interplay between proteolytic degradation pathways*

17:30-17:50 – **Laurent NOËL** ***(CNRS, FR):*** *Time-lapse analysis of* Xanthomonas *infecting cauliflower hydathodes*

17:50-18:00 – **Wrap-up of Session 2**

Thursday 14th of December

Session 3 Genetic Resistance – Host Defence

Chairs: Jens BOCH & Roland KÖLLIKER

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8:30-10:30 **– *Social Event (Visit of old university)***

10:30-11:00 – COFFEE BREAK

11:00-11:30 – **Panagiotis SARRIS *(University of Exter, UK):*** *Plant immune receptors mimic pathogen virulence targets*

11:30-11:50 – **Bharat Bhusan MAJHI *(University of Tel Aviv, IS)*:** *BSK5 is a component of PTI signaling, and its tomato homolog BSK830 interacts with multiple* Xanthomonas euvesicatoria *effectors*

11:50-12:10 – **Florent DEPLACE *(INRA, FR)*:** *RKS1, a starting point to decipher the gene regulatory network controlling quantitative disease resistance against* Xanthomonas campestris

12:10-12:30 – **Norman ADLUNG *(Martin-Luther-Universität Halle-Wittenberg, DE):*** *Dissecting virulence function from recognition: cell death suppression in* Nicotiana benthamiana *by XopQ/HopQ1-family effectors relies on EDS1-dependent immunity*

12:30-14:00 – LUNCH

14:00-14:20 – **Harold VAN DEN BURG *(Amsterdam University, NL)***: *Guttation induction as means to study closely the natural infection of Arabidopsis thaliana by* Xanthomonas campestris *pv.* campestris

14:20-14:40 – **Maria LENOR CRUZ *(Instituto Nacional de Investigação Agrária Veterinária, PT):*** *New insights on Black Rot of Crucifers: Disclosing novel virulence genes by in vivo host/pathogen transcriptomics and functional genetics*

14:40-15:00 – **Grazia LICCIARDELLO** ***(University of Catania, IT):*** *Resistance evaluation of Citrus ornamental relatives to* Xanthomonas citri *pathovars and pathotypes causal agent of Citrus Bacterial Canker*

15:00-15:20 – **Marieke VAN HUELTEN** ***(University of Amsterdam, NL)*****:** *Genetic basis for basal resistance against* Xanthomonas campestris *pv. campestris in* Arabidopsis thaliana

15:20-15:30 – **Wrap-up of Session 3**

15:30-16:00 – ***Presentation of Activities of the EuroXanth COST Action & Plenary Discussion***

16:00-16:30 – COFFEE BREAK

16:30-18:00 – ***Poster Session***

16:30-18:00 – ***COST Management Committee Meeting***

19:30 – Walk through the historical center of Coimbra to the restaurant

20:00 – GALA DINNER

Friday 15th of December

Session 4 Disease Management – Vector Control

Chairs: Maria SAPONARI & Tamas KOVACS

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9:15-9:45 – **Juan NAVES CORTES *(IAS, ES)*:** *Climatic suitability for* Xylella fastidiosa *using species distribution models*

9:45-10:05 – **Edyta ĐERMIĆ *(University of Zagreb, HR)*:** *On preparations for timely detection of* Xylella fastidiosa *in Croatia*

10:05-10:25 – **Massimilliano MORELLI *(CNR-Institute for Sustainable Plant Protection, IT)*:** *Evaluating biocontrol of* Xylella fastidiosa *disease in olive with a beneficial endophyte*

10:25-10:45 – **Eliška PEŇÁZOVÁ *(Mendel University, CZ):*** *Susceptibility testing of cabbage breeding lines to black rot infection*

10:45-11:15 – COFFEE BREAK

11:15-11:35 – **Nicholas SKANDALIS *(Institute of Molecular Biology and Biotechnology, GR):*** *Biotic and abiotic stress extends the target range of the commercial* Bacillus amyloliquefaciens *strain MBI600 and upregulates antibiotic production and competence*

11:35-11:55 – **Shiri TOPMAN *(Department of Plant Pathology and Microbiology, IS):*** *Potential of random peptide mixtures as crop protection agents against plant diseases caused by* Xanthomonas *and other plant pathogenic bacteria*

11:55-12:15 – **Jakub PEČENKA *(Mendel University, CZ):*** *Antibacterial effect of selected nanoparticles as revealed by doubling time of treated* Xanthomonas campestris *pv.* campestris *cultures*

12:15-12:25– **Wrap-up of Session 4**

12:25-12:45 – ***Concluding remarks by the organisers***

12:45-14:15 – LUNCH

Session 1

Diagnostics & Diversity – Population Structure

Chairs: Joana COSTA & Nicholas SKANDALIS

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Is *Xylella fastidiosa* really emerging in France?

Nicolas Denancé[1,2], Sophie Cesbron[1], Martial Briand[1], Adrien Rieux[3] and **Marie-Agnès Jacques**[1]

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Keywords: MLSA, comparative genomics, quarantine disease

Listed as a quarantine pest worldwide except in the Americas, *Xylella fastidiosa* was first detected in Europe very recently with reports from Italy in 2013, France in 2015, Germany and Spain in 2016. A large genetic diversity has been evidenced in these different outbreaks, with representatives from at least the three main subspecies (*i.e. fastidiosa, multiplex,* and *pauca)*. In France, most foci are consecutive to *X. fastidiosa* subsp. *multiplex* ST6 and ST7. However, foci of *X. fastidiosa* subsp. *pauca* ST53 *X. fastidiosa* subsp. *fastidiosa/sandyi* ST76 and *X. fastidiosa* subsp. *multiplex* ST79 have been detected. Molecular dating of phylogenetic trees exploiting genome data provided dates of divergence between French isolates and their American relatives that can be considered as proxy or lower bounds of introduction dates. According to these analyses, *X. fastidiosa* may have been introduced in France as early as 1965 (ST7) and 1980 (ST6). These dates are also coherent with increases in introduction of alien plant material in Corsica. Altogether these results and the various detections of foci of genetically diverse *X. fastidiosa* strains in Europe support the idea that *X. fastidiosa* has been introduced in Europe in separate occasions since ~50 years with apparent diverse epidemiological patterns and socio-economic consequences.

Identification and distribution of prevalent races of *Xanthomonas campestris* pv. *campestris* in Italy

Patrizia Bella[1], Chiaraluce Moretti[2], Grazia Licciardello[3], Cinzia P. Strano[3], Massimo Zaccardelli[4], Ferdinando Branca[3], Roberto Buonaurio[2], Joana G. Vicente[5] and **Vittoria Catara**[3]

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Keywords: Black rot of *Brassicaceae*, population diversity, race typing, MLSA

Black rot caused by *Xanthomonas campestris* pv. *campestris* (*Xcc*) is the most important disease of *Brassicaceae*. Nine races were identified based on their interaction with differential *Brassica* lines. Knowledge of the frequency and distribution of these pathogenic variants within *Xcc* populations is necessary to set up effective resistance breeding programs. In Italy, the disease is present in all regions and severe outbreaks were reported affecting up to 100% of *Brassica* plants both in production of crops and ornamentals, but no studies on race structure have been carried out. Thirty-one *Xcc* strains, isolated from six *Brassica oleracea* varieties, *B. napus* var. *napobrassica* and *Crambe maritima* in seven Italian regions were selected from a larger collection for further study. Strains were characterised by MLSA based on the sequences of four conserved protein-coded genes (*dnaK*, *rpoD, fyuA,* and *gyrB*) and were inoculated on eight differential *Brassica* lines and the phenotype interaction assessed after seven and 15 days. The race 4 was predominant within Italian *Xcc* strains (54.8 %), followed by race 1 (32.2 %). These two races were widespread in six out of seven Italian regions. Only four Italian *Xcc* strains were characterized as race 6 (12.9 %) and were identified only in three regions. Generally, no correlation was found between race, geographical origin, host of isolation and MLSA results of the 31 *Xcc* strains. Interestingly, seven out of eight *Xcc* strains isolated in three different regions from *B. oleracea* var. *gongylodes* belonged to race 4.

Xanthomonas arboricola pv. *juglandis*: an endemic walnut pathogen in Portugal

Camila Fernandes[1,2,3], Pedro Albuquerque[1], Leonor Cruz[2,4] and Fernando Tavares[1,3]

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Keywords: dot blot hybridization, DNA markers, epidemiology, MLSA, *Xanthomonas arboricola*

Xanthomonas arboricola pv. *juglandis* (*Xaj*) is one of the most serious and widespread threats of walnut orchards, associated with severe production losses having a large negative economic impact. These concerns made *Xaj* the focus of several diversity studies to infer epidemiological patterns and contribute to improve phytosanitary practices. Regardless the high genetic diversity of *Xaj* emphasized in previous studies, comprehensive assessments of *Xaj* population diversity taking into account multiple variables thought to modulate the disease ecology of these bacteria are still lacking. To tackle this issue, we assess the genetic diversity of 131 *Xaj* isolates obtained from leaves, fruits, branches, catkins and buds of symptomatic walnut trees distributed throughout distinct climatic regions of Portugal, between 2014 and 2016. Isolates' diversity was assessed using a dot blot hybridization platform with nine *Xaj*-specific DNA markers and by multilocus sequence analysis (MLSA) using four housekeeping genes.

The results showed that *Xaj* lineages present in walnut-growing regions of Portugal are genetically heterogeneous, distributed by ten clusters (I to X) inferred by the Maximum Likelihood analysis of 248 concatenated sequences (*acnB*, fyuA, *gyrB* and *rpoD*) and 20 different hybridization patterns. Two clusters (IX and X) were shown to diverge from the main *Xaj* lineages, and different *Xaj* lineages were isolated from the same walnut tree. The present study suggested that *Xaj* diversity is not dependent on bioclimatic regions, walnut cultivars, plant organ or isolation date, which is evocative of a cosmopolitan dispersion.

Molecular epidemiology of *Xanthomonas euvesicatoria* strains from the Balkan Peninsula revealed by a new multiple-locus variable-number tandem-repeat analysis scheme

Taca Vancheva[1,2], Nevena Bogatzevska[3], Penka Moncheva[1], Ralf Koebnik[2] and **Christian Vernière**[4]

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Keywords: Bacterial spot, population structure, genetic diversity, size homoplasy

Molecular epidemiology is useful to describe the inoculum sources, the transmission pathway or dispersal ability of pathogens and then to improve control methods. It is based on genetic markers whose variation contributes to discriminate between individuals or populations. Short arrays of tandem repeats (TR) organized at distinct loci show high mutation rates producing variable numbers of TR (VNTR). After screening 12 genome sequences of different pathovars of *Xanthomonas euvesicatoria*, we developed a Multi-Locus-VNTR Analysis scheme targeting 16 TR loci (MLVA-16) that were directly assessed by sequencing. We applied this MLVA scheme to a collection of pepper-pathogenic strains of *X. euvesicatoria* from Bulgaria and Macedonia to trace back the spread and the genetic structure of these strains. Bacterial spot has been reported in Bulgaria and Macedonia during the first part or at the end of the XXth century, respectively. Thirty-six VNTR haplotypes out of 88 strains were resolved with four haplotypes found in both countries. Allelic richness was higher in Bulgaria with a much greater private allelic richness. A minimum spanning tree from the VNTR dataset separated the haplotypes in 8 clonal complexes (CCs) and 10 singletons. Four CCs grouped strains from the two countries. Sequence variation occurred for a few identical alleles within 3 TR loci generating size homoplasy. It allowed separating two genetic lineages present in both countries. Our data suggest multiple introduction events in both countries with some epidemiological link between Bulgaria and Macedonia *X. euvesicatoria* strains.

Genomic-based development of a real-time PCR protocol for improving the diagnosis of *Xanthomonas arboricola* pv. *pruni*, the causal agent of bacterial spot disease of *Prunus* spp.

Jerson Garita-Cambroner[1,2], Ana Palacio-Bielsa[3] and **Jaime Cubero**[1]

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Keywords: *Xap*, bacterial spot of stone fruits, quarantine

Comparative genomics of *Xanthomonas arboricola* species has unveiled a detailed view of the instraspecific evolutionary history of this plant-pathogen as well as a characterization of its pathogenic arsenal associated with the disease process. In addition to these analyses, phenotypic and pathogenic assays have revealed the presence of non-pathogenic strains which are phylogenetically different to those pathogenic strains of the pathovars *pruni*. Comparative analyses among these two groups showed up a wide range of genomic regions that could be useful to differentiate these two intraspecific groups that cohabit on *Prunus*. Taking advantage of the variation in this genomic feature, a real-time PCR protocol, based on a partial sequences of the *xopE3* gene, has been developed to differentiate *Prunus*-pathogenic and non-pathogenic strains of *X. arboricola* and to refine the diagnosis of this quarantine pathogen in the EU. The use of this new protocol in conjunction with a previous protocol based in the amplification of the gen *ftsX* showed a high specificity to differentiate pathovar *pruni* from the other instraspecific groups of *X. arboricola* as well as a high sensitivity (100 cfu/ml or 100 pg/µl of DNA) and efficiency (1.8-2.0, being 2.0 a 100% of efficiency). This new protocol is a valuable molecular tool for the improvement of the diagnosis of the causal agent of bacterial spot of stone fruits and almond.

Development of new diagnostic tools based on the type III secretion system of Gram$^-$ phytopathogenic bacteria

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Keywords: diagnostic microarray, Gram negative, type III secretion system

We developed alternative diagnostic tools based on in silico analysis of core components of the type III secretion system (T3SS), a protein export system of Gram-negative bacteria which forms a pilus/injectisome that translocates effector proteins into eukaryotic host cells. T3SS core DNA sequences from more than 100 pathogenic strains spanning ten bacterial genera were used for the phylogenetic reconstruction of the specific genomic region and the design of a diagnostic microarray. More than 800 pathovar-specific probes were generated and spotted with high replication on a custom 8x15K chip. Probes were Cy3-labeled and hybridized with samples from 16 different preparations that involved either total bacterial DNA or total DNA from plants infected with bacterial pathogens. Eleven different pathovars were represented in those 16 preparations. Consistently with phylogenetic affiliations, hierarchical clustering of the normalized intensities of all probes produced clear groupings of samples with high statistical significance. Mixed samples revealed identical patterns with their pure counterparts, despite the existence of plant genetic background. Using linear modelling and empirical Bayes statistics, unknown samples from known hosts could be safely classified and identified. Finally, by studying the distribution of the successfully designed probes along the T3SS core, a guideline for the diagnostic use of this specific region by several other approaches was provided and confirmed by a small-scale microarray and multiplex qPCR.

Guidelines to discover new xanthomonads species-specific markers for detection and genotyping: from the in silico analysis to experimental validation

Fernando Tavares[1,2], Pedro Albuquerque[1] and Camila Fernandes[1,2,3]

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Keywords: DNA markers, DNA signatures, PCR, dot-blot hybridization, xanthomonads

We are now in the post-genomics era, where the affordability of high-throughput sequencing provided by NGS platforms, the massive amount of data in genebanks, including the thousands of bacteria genomes available, and the convenience of ingenious bioinformatics tools, fostered the ability to rapidly survey bacterial genomes and identify highly discriminatory DNA-signatures, i.e. taxa-specific markers (nucleotide sequences that can be used to detect the presence of certain organisms and to distinguish from all other species – Phillippy et al. 2009).

In these last few years our research group has been working to select and validate species-specific markers for several bacteria, including xanthomonads, which allow conciliating the specificity of DNA-detection assays with the clonal resolution of genotyping, due to their allelic variation. Although these markers have been mainly used for diagnostics, their utility to access uncultured bacteria may help to delineate more comprehensive and truthful diversity studies. The methodology to identify DNA signatures consisted essentially on bioinformatics tools namely CUPID, Insignia, blast surveys, and a dedicated in silico pipeline, which resulted in the identification of numerous novel species-specific markers for several xanthomonads (Fernandes et al. 2017, Albuquerque et al. 2012). Experimental validation by PCR methods and dot-blot hybridization, confirm the high specificity and efficiency of these markers for culture-independent detection methods, and their genotyping utility to access the population diversity of bacteria.

Postgenomic lateral transfer knowledge from *Salmonella enterica*, *Mycobacterium tuberculosis, Legionella pneumophila* CRISPRs genetic diversity knowledge and expertise to a new model: *Xanthomonas oryzae*

Christophe Sola[1], Clément Ripoll[2] and Guislaine Refrégier[1]

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Keywords: CRISPR, genetic diversity, SNPs, multiplexing analysis

Based on previous experience and knowedge acquired during 10 years of postgenomic integrated research on *Mycobacterium tuberculosis* and on *Salmonella enterica* CRISPR genetic diversity, we propose a transposition of this approach towards the description of population structure, new diagnostics method development and new surveillance tools applicable to *Xanthomonas* CRISPR population structure knowledge and diagnostics.

This will include *in Silico* (database building and data-mining), *in Vitro* (new method development), and geographic information system building, to try to improve surveillance of this economically important pathogen agent or to decipher its population structure.

We report on the data-mining methodology we used, the methods we developed, and describe recent results obtained using CRISPR diversity studies on *Mycobacterium tuberculosis*, *Legionella pneumophila* and *Salmonella enterica* as study models. We discuss how some of this previous knowledge and acquired expertises could be transferred to *Xanthomonas* studies, either for academic or for applied purposes.

Present situation and study on *Xanthomonas arboricola* in Poland

Monika Kałużna and Joanna Puławska

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Since few years the presence of symptoms indicating blight on walnut and hazelnut were reported in Poland. Selected phenotypic characteristics and pathogenicity test done for isolates obtained from diseased tissue confirmed the presence of *X. a.* pv. *juglandis (Xaj)* and *X. a.* pv. *corylina* (*Xac*). Results of PCR MP, ERIC- BOX-PCR, and MLST indicated similarity between the studied isolates and the reference strain of *Xaj* CFBP 7179; except, strains I-391 and LMG 746. MLST analysis of partial sequences of the 3 genes showed that the *Xaj* population consists of different phylogenetic lineages. An incongruence among MLST gene phylogenies and traces of intergenic recombination events were observed.

Results of majority of the physiological and biochemical features determined for isolates obtained from hazelnut agreed with those given in the EPPO standard PM 7/22, but some of them differed. The isolates tested were also identified as *X. a.* pv. *corylina* (*Xac*) on the basis of (FAME) as well as in *gyrB* gene sequence analysis. In rep-PCR all isolates showed patterns very similar to reference *Xac* strains.

Although the symptoms resembling bacterial spot caused by *X. a.* pv. *pruni* are observed every year, so far this pathogen was not isolated from diseased tissue.

The further study on *Xanthmonas arboricola* pvs. are planned to be conducted within a national project in frame of COST CA 16107.

Characterisation of new species of *Xanthomonas* associated with watercress

Joana G. Vicente[1], Steve D. Rothwell[2], Eric B. Holub[1] and David J. Studholme[3]

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Keywords: black rot, wilt, pathogenicity, sequencing, *Brassicaceae*

Watercress (*Nasturtium officinale*) is a semi-aquatic *Brassicaceae* used as a salad crop. Black rot bacterial disease has been occasionally reported in watercress, but the pathogen that cause this disease had not been described. Strains of *Xanthomonas* and *Pseudomonas* spp. were isolated from diseased leaves of watercress (*Nasturtium officinale*) produced in Florida, USA. Pathogenicity tests in a range of hosts showed that one strain was pathogenic on watercress, but not in any other plants whilst all other strains were not pathogenic. Data from Biolog carbon source utilization tests and nucleotide sequence data from 16S and *gyrB* loci from the *Xanthomona*s strains showed that both pathogenic and non-pathogenic strains were distinct from previously described species. Multilocus sequence analysis and whole genome-wide comparisons of average nucleotide identity (ANI) of genomes of two strains from watercress showed that these are distinct and share less than 95% ANI with all other known species. Two new *Xanthomonas* species named *X. floridensis* sp. nov. (type strain WHRI 8848, non-pathogenic) and *X. nasturtii* sp. nov. (type strain WHRI 8853, pathogenic on wallflower) have been proposed. This is the first description of a pathogen that cause black rot or wilt of wallflower and, interestingly, the first report of a *Xanthomonas* disease of a *Brassicaceae* that is not caused by bacteria of the *X. campestris* species. The possible interactions of pathogenic and non-pathogenic *Xanthomonas* strains co-existing in the same host should be further studied. The importance of the disease in watercress production is still unknown and should be investigated.

Session 2

Pathogen Biology

Chairs: Joël F. POTHIER & Guido SESSA

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Comparative genomics of the order *Xanthomonadales*: a glimpse of pathogenicity evolution of *Xanthomonas* and *Xylella* genera

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Keywords: virulence, type 3 secretion system, effector, cell wall degrading enzymes, CUT loci

The orders *Xanthomonadales, Nevskiales* and *Cardiobacteriales* occupy a specific taxonomic position at the base of the class Gammaproteobacteria. We have undertaken a comparative genomic analysis at the level of these three orders to identify molecular determinants allowing bacterial adaptation to plants and to study the evolution of pathogenicity of *Xanthomonas* and *Xylella*. A total of 443 genomes covering the 3 orders have been selected for their quality and have been re-annotated, using the same pipeline. More than 25 000 groups of orthology have been defined and used to perform our comparative analysis. Beside already known virulence factors, this work allowed the identification of new specific functions which might be involved in the adaption to plants and pathogenicity of *Xanthomonas* and *Xylella*.

Xanthomonas redirects host defense metabolism to benefit infection

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Keywords: *Xanthomonas oryzae* pv. *oryzae*, TALE, rice

Transcription activator-like effectors (TALEs) are bacterial proteins that operate as transcription factors in eukaryotic cells. Plant-pathogenic *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) use a large number of TALEs to manipulate host plant cells and cause bacterial leaf blight, a devastating disease of rice. TALE proteins bind DNA via 34-amino acid repeats with each repeat containing an RVD (repeat variable diresidue), which recognizes one base in the target DNA.

Xoo strains from the Philippines and India were sequenced to investigate differences and similarities between TALE gene repertoires of *Xoo* strains from independent locations. Potential target genes of each TALE were predicted with AnnoTALE and TALE-mediated gene induction was analyzed using RNA-seq of infected rice plants.

Artificial TALEs with RVD compositions identical to their natural counterparts were constructed and introduced into an *Xoo* strain without natural TALEs. Strains expressing individual TALEs were used to verify gene induction of potential target genes in rice via qRT-PCR.

Rice promoters of identified TALE target genes were cloned upstream of a *uidA* reporter gene to investigate whether the corresponding TALEs can induce them directly. The reporter constructs and TALE expression constructs were co-delivered into *Nicotiana benthamiana* via *Agrobacterium tumefaciens* and GUS staining, and quantitative GUS assays were performed.

With this approach, it is possible to reliably determine TALE target genes. We identified a new potential susceptibility target for *Xoo* which indicates that the pathogen manipulates the host metabolome to redirect defense pathways and to support an infection.

Transcriptome analysis of *Xanthomonas fragariae* in strawberry plants with application of RNA-seq

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Xanthomonas fragariae, which causes angular leave spot on strawberry, is a quarantine organism that is a reason of strawberry damage worldwide. The purpose of our study was to analyze the mechanism of interaction of bacterium with plant on the transcriptome level. For this purpose, mRNA of *X. fragariae* growing on the TY microbial medium and from the infected Elsanta strawberry plants were isolated and sequences on the Illumina MiSeq platform.

Expression profiles of bacteria on TY medium and *in planta* were very diverse. Of the 3939 CDSs recorded, 1995 had significantly different expression *in planta* (966 genes were down and 1029 up-regulated). Among the genes of increased expression *in planta* eggNOG/COG categories associated with bacterial cell motility, signal transduction, transport and metabolism of inorganic ions and carbohydrates and transcription were overrepresented. Among the genes with the most increased expression *in planta* primarily genes associated with flagella synthesis and chemotaxis were found. It is also interesting to note that out of the 31 genes localized on the plasmid, 16 were expressed differently *in planta*, which may indicate their potential role in plant-pathogen interaction. Among genes with differentiated expression localized both on chromosome and plasmid, there are many that encode proteins of unknown function.

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Genetics of bacterial fitness of *X. campestris in vitro* and *in planta*

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Keywords: *Xanthomonas*, fitness, virulence, plant adaptation

Xanthomonas campestris pv. *campestris* (*Xcc*) is the causal agent of black rot disease on cultivated or wild Brassicaceae. So far, genetic screens have been qualitative and only identified genes essential for pathogenicity or avirulence. Mechanisms of pathogen entry, vascular immunity and microbial fitness in plant environments are essentially unknown. Through the recent advances in sequencing techniques, numerous bacterial genomes are now available. However, the functions of many genes remain uncharacterized. To bridge the gap between the identification and functional characterization of genes, high-throughput experimental approaches are required. In order to identify the pathways important for bacterial fitness at different steps of plant infection and in different *in vitro* and *in planta* conditions, we have initiated a study using TnSeq a highthroughput screen of barcoded transposon (Tn) mutants of *Xcc.* TnSeq approach evaluates bacterial fitness by counting the relative mutant abundance of a barcoded library of Tn mutants using Next Sequencing Generation. It has successfully been used over the past years to identify genes required for the virulence of numerous human bacterial pathogens. The barcoded library of Tn mutants of strain 8004 (ca. 500.000 independent insertions) was produced and initially characterized. TnSeq is a useful tool to identify important genes for bacterial fitness (metabolic capacities, epiphytic survival, plant mesophyll and vascular colonization, defense evasion, interactions with the microbiome, seed infection, abiotic stress tolerance) and open novel avenues of research. Recent results will be presented.

How *Xanthomonas* hijacks the interplay between proteolytic degradation pathways

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Autophagy and the ubiquitin-proteasome system (UPS) are the major pathways for protein degradation in eukaryotes. They orchestrate many cellular processes during development and in response to environmental stimuli such as microbial infections. In plants, the contribution of the UPS to immune responses and its targeting and exploitation by pathogens are well documented, but the role of autophagy remains elusive. Recent advances in the plant-microbe interaction field revealed pro- and anti-microbial roles of autophagy. Here, we show that the T3E XopL of *Xanthomonas campestris* pv. *vesicatoria*, causal agent of bacterial spot of pepper and tomato, possibly modulates autophagy responses by interacting with an autophagy component. *In planta* interaction studies revealed that both proteins interact in vesicle-like mobile structures in the host cytoplasm. Given the fact that XopL constitutes a novel class of E3 ligases, we propose that XopL might interfere with autophagy via ubiquitylatation and proteasome-dependent degradation of its interaction partner. Preliminary results of the possible effect of XopL on its target indicate that XopL degrades this autophagy component in a proteasome-dependent manner. To assess whether the possible XopL-mediated degradation of an autophagy component impairs autophagy responses, we developed a dual-luciferase based quantitative autophagy assay. Using this screen for autophagy modulation we identified that XopL might affect autophagic turnover, while other *Xcv* T3Es possess different activities. Taken together, we suggest that T3E XopL exploits the UPS to degrade an autophagy component leading to altered autophagy responses.

Time-lapse analysis of *Xanthomonas* infecting cauliflower hydathodes

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Hydathodes are water pores found on leaves of a wide range of vascular plants and are the sites of guttation. We report here on the detailed anatomy of cauliflower and Arabidopsis hydathodes. Hydathode surface presents pores resembling stomata giving access to large cavities. Beneath, the epithem is composed of a lacunar and highly vascularized parenchyma offering a direct connection between leaf surface and xylem vessels. Arabidopsis hydathode pores were responsive to ABA and light similar to stomata. The flg22 flagellin peptide, a well-characterized elicitor of plant basal immunity, did not induce closure of hydathode pores in contrast to stomata. Because hydathodes are natural infection routes for several pathogens, we investigated hydathode infection by the adapted vascular phytopathogenic bacterium *Xanthomonas campestris* pv. *campestris* (*Xcc*), the causal agent of black rot disease of *Brassicaceae*. Microscopic observations of hydathodes six days post inoculation indicated a digestion of the epithem cells and a high bacterial multiplication. Post-invasive immunity was shown to limit pathogen growth in the epithem and is actively suppressed by the type III secretion system and its effector proteins. Altogether, these results give a detailed anatomic description of *Brassicaceae* hydathodes and highlight the efficient use of this tissue as an initial niche for subsequent vascular systemic dissemination of *Xcc* in distant plant tissues.

Session 3

Genetic Resistance – Host Defence

Chairs: Jens BOCH & Roland KÖLLIKER

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Plant immune receptors mimic pathogen virulence targets

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Keywords: NLR immune receptors, effectors recognition, plant immunity, durable resistance

The plant innate immunity relies on the presence of specific immune receptors like the cytoplasmic NLR (Nucleotide binding-Leucine rich Repeat) receptors. The plant NLRs structurally and functionally resemble mammalian Nod-Like Receptors and accomplish intracellular detection of pathogen effectors, either directly or indirectly. The result of this recognition is the activation of an immune signalling pathway known as Effector Triggered Immunity (ETI) that often culminates in a Hypersensitive cell-death Response (HR). Like in animals, the plant NLR receptors have a modular structure and can work in pairs, both of which are required for defense activation upon recognition of specific pathogen effectors. However, how such intracellular immune receptor complexes activate defense solely upon recognition of microbial molecules is poorly understood. We recently demonstrated that NLRs with non-canonical domain architectures play an important role in plant immunity. These composite immune receptors are thought to arise from fusions between NLRs and additional domains that serve as “baits” or “decoys” for the pathogen-derived effector proteins, thus enabling pathogen recognition. Several names have been proposed to describe these proteins, including “integrated decoys” and “integrated domains”. In order to investigate the presence of NLRs with “integrated domains” (NLR-IDs) in various plant species, we have scanned available plant genome sequences for the full spectrum of NLR-IDs to evaluate the diversity of integrations of potential IDs across flowering plants, including economically important crops.

BSK5 is a component of PTI signaling, and its tomato homolog BSK830 interacts with multiple *Xanthomonas euvesicatoria* effectors

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Keywords: BSK, effector, PTI, *Xanthomonas euvesicatoria*

Brassinosteroid-signaling kinases (BSKs) belong to the receptor-like cytoplasmic kinase XII sub-family. BSK5 was found to interact with several pattern-recognition receptors (PRRs), including flagellin-sensitive-2 (FLS2), EF-Tu receptor (EFR), and PEP-receptor1 (PEPR1) in yeast and *in planta*. Importantly, BSK5 was phosphorylated *in vitro* by PEPR1 and EFR. The *bsk5* T-DNA insertion mutant was compromised in immunity to the fungal pathogen *Botrytis cinerea* and to *Pseudomonas syringae* pv. *tomato* bacteria. Moreover, the mutant was impaired in defense responses associated with pattern-triggered immunity (PTI), including generation of reactive oxygen species and callose deposition at the cell wall, but was not affected in activation of MAP kinases. Similarly, the tomato homolog BSK830 interacted with the PRRs FLS3, SlFLS2 and BTI9. To test the hypothesis that *Xanthomonas euvesicatoria* (*Xe*) type III effectors target tomato components of PTI signaling, we tested the interaction of 35 *Xe* effectors with PRRs and BSK830 *in planta*. None of the effectors interacted with any of the tested PRRs, while seven effectors (XopAE, XopAW, XopB, XopC, XopG, AvrRxo1 and XopX) interacted with BSK830. Our results suggest that BSKs are components of PTI signaling and tomato BSK830 may be a target of multiple *Xe* effectors.

RKS1, a starting point to decipher the gene regulatory network controlling quantitative disease resistance against *Xanthomonas campestris*

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Keywords: quantitative disease resistance, signalling pathways, transcriptomics, protein-protein interactions, *Arabidopsis thaliana*, *Xanthomonas campestris*

The importance of pathogen perception and signaling pathways in the regulation and execution of plant immune responses have become apparent during the last years. Notably, *R* gene-mediated immunity has been shown to be the most efficient form of resistance in plants, but it is also not durable. Additional forms of resistance have gained increasing attention for breeding purposes, such as quantitative resistance that is considered a more durable type of resistance with partial effects on plant immunity. The identification of genes underlying QDR might have practical implications to increase crop yield and quality. However, there is still very limited information about the molecular mechanisms controlling QDR. One of the most important disease of crucifers (including Cabbage, Broccoli and Rapeseed), is the black rot, caused by the bacterial plant pathogen *Xanthomonas campestris* pv. *campestris* (*Xcc*). Based on results obtained by genome wide associating (GWA) mapping approach, we were able to identify Resistance related KinaSe 1 (RKS1) as the responsible gene underlying a major QTL conferring approximately 50% resistance in *Arabidopsis thaliana* against the strain *Xcc568* (Huard-Chauveau et al., 2013). Our study aims to decipher the regulatory pathway(s) controlling and controlled by RKS1. We used two complementary approaches: (i) comparative transcriptome analysis of mis-expressing *RKS1* lines, and (ii) identification of proteins interacting with RKS1 by Yeast-two-Hybrid assays. Results and perspectives of this work will be presented.

Dissecting virulence function from recognition: cell death suppression in *Nicotiana benthamiana* by XopQ/HopQ1-family effectors relies on *EDS1*-dependent immunity

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Many Gram-negative plant pathogenic bacteria express effector proteins of the XopQ/HopQ1 family which are translocated into plant cells via the type III secretion system. In *Nicotiana benthamiana*, recognition of XopQ/HopQ1 proteins induces an effector-triggered immunity (ETI) reaction which is not associated with strong cell death but renders plants immune against *Pseudomonas syringae* and *Xanthomonas campestris* pv. *vesicatoria* strains. Additionally, XopQ suppresses cell death in *N. benthamiana* when transiently co-expressed with cell death inducers. Here, we show that representative XopQ/HopQ1 proteins are recognized similarly, likely by a single resistance protein of the TIR-NB-LRR class. Extensive analysis of XopQ derivatives indicates the recognition of structural features and by direct interaction. We performed *Agrobacterium*-mediated protein expression experiments in wild-type and *EDS1*-deficient (*eds1*) *N. benthamiana* leaves, not recognizing XopQ/HopQ1. XopQ recognition limits multiplication of *Agrobacterium* and attenuates levels of transiently expressed proteins. Remarkably, XopQ fails to suppress cell death reactions induced by different effectors in *eds1* plants. We conclude that XopQ-mediated cell death suppression in *N. benthamiana* is due to the attenuation of *Agrobacterium*-mediated protein expression rather than the cause of the genuine XopQ virulence activity. Thus, our study expands our understanding of XopQ recognition and function, and also challenges the commonly used co-expression assays for elucidation of in planta effector activities, at least under conditions of ETI induction.

Guttation induction as means to study closely the natural infection of *Arabidopsis thaliana* by *Xanthomonas campestris* pv. *campestris*

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Xanthomonas campestris pv. *campestris* (*Xcc*), the causal agent of Black rot in crucifers, is one of the few bacterial pathogens that enters its host via hydathodes. These specialized organs on the leaf margins are in direct contact with the leaf vasculature. The hydathode cavity contains a tissue layer, the epithem, which forms the only natural barrier for bacterial ingress in the xylem, while allowing passage of xylem sap when the root pressure is high. Recent studies have shown that *Xcc* enters the xylem by dissolving the epithem in a type III secretion-dependent manner. Hence, the arms-race between *Xcc* and crucifers occurs at this very initial stage of infection, in which the epithem apparently can ward off invading bacteria in an incompatible interaction. In the compatible interaction, *Xcc* can dissolve the epithem, colonize the xylem, and ultimately block the water flow inside vessels due to biofilm formation (resulting in the typical V-shaped wilting and chlorotic symptoms). In the past, studies on resistance against *Xcc* have primarily focused on *Xcc* recognition inside the xylem. To this end, bacteria were directly pricked into the vasculature by wounding the midvein or clipping the leaf ends using contaminated scissors. Other groups have studied *Xcc* infection by infiltrating bacterial suspensions into the leaf apoplast- a tissue in which *Xcc* is normally not detected. We have now developed a 'hydathode guttation'-based entry assay for *Xcc* in the model plant *Arabidopsis thaliana.* With this assay, we can determine the genetic basis for the initial interaction between *Xcc* and *A. thaliana*. Our assay uses spray-inoculation in combination with guttation induction. Our conditions then stimulate bacterial entry via re-uptake of guttation droplets. With this method we study the main mechanisms that allow *Xcc* ingress and/or provide resistance to the host plant. To perform genetic screens, we have optimized a bioluminescent reporter strain of *Xcc*, which allows us to follow bacterial invasion via hydathodes and subsequent systemic spread in the vasculature. Since *Xcc* proliferates to high densities very locally in only a subset of xylem vessels, this has the advantage over classical colony-count methods. Using this improved bioassay we are currently screening Arabidopsis populations in search of new resistance mechanisms against *Xcc*.

New Insights on Black Rot of Crucifers: Disclosing novel virulence genes by *in vivo* host/pathogen transcriptomics and functional genetics

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Keywords: Black rot disease, phenetic characterization, RNA-Seq, Virulence regulation

Xanthomonas campestris (*Xc*) presently comprises pathovars *campestris* (*Xcc*), *raphani* and *incanae*, affecting Brassicaceae and some Solanaceae plants. Although studies on the interaction of *Xcc* with *B. oleracea* have identified several virulence genes, no resistance genes have been successfully cloned in Brassicaceae crops and there is still a lack of control measures. Molecular mechanisms of host-pathogen interaction have been inferred using *in vitro* strategies. With the goal of bringing new insights on the molecular mechanisms of host/pathogen interaction, a set of 33 *Xc* strains collected in Portugal was characterized, towards pathogenicity, virulence, population structure and phylogenetic diversity. Two new *Xcc* races were described and the high level of phenotypic diversity was supported by phylogenetic data, correlating the three pathovars with three distinct genetic lineages. Virulence assessment, after determining percentage of infected leaf area, allowed selecting the highest and lowest virulence strains. *In planta* transcriptomes of selected *Xcc* strains during the infection process on two selected hosts were profiled using RNA-Seq, in a total of four pathosystems. This approach revealed that *Xcc* undergoes a host-independent transcriptional reprogramming, involving 154 differentially expressed genes, including Type III effectors, detoxification and cell wall degrading enzymes coding genes. The study of differential infection process *in planta* using this innovative strategy contributed to disclose novel virulence related genes in *X. campestris* pv. *campestris* - *B. oleracea* pathosystem, crucial for developing new tools for black rot disease control.

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Resistance evaluation of Citrus Ornamental Relatives to *Xanthomonas citri* pathovars and pathotypes causal agent of Citrus Bacterial Canker

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Keywords: canker lesions, leaf assay, susceptibility, bacterial population

Xanthomonas citri pv. *citri* (*Xcc*) and *X. citri* pv. *aurantifolii* (*Xca*) are causal agents of Citrus Bacterial Canker (CBC) and of quarantine concern for EU. As reported by EFSA (2014), the susceptibility of ornamental rutaceous species other than *Citrus*, *Fortunella* and *Poncirus* to these pathogens and the associated symptomatology, has not been fully assessed. In the framework of the research project ORPRAMed, we have evaluated, by a detached leaf assay, the resistance of 25 Citrus related ornamental plants in the genera *Atalantia, Balsamocitrus*, *Clausena*, *Eremocitrus*, *Glycosmis*, *Melicope*, *Microcitrus*, *Murraya, Vespris*, as well as a number of *Citrus* and *Fortunella* ornamentals, to *Xcc* (pathotype A, A*, A^w) and *Xca* (pathotype B and C) strains (one strain per lineage). Interestingly, variable results were obtained within the same genus, spanning from the high susceptibility of *M. ovatifoliolata* to the resistance of *M. paniculata* and *M. koenigii* to all pathotypes. High variability was also detected in *Fortunella* ranging from the non-host status of *F. margarita* to all pathotypes and the susceptibility of *F. hindisii* to pathotype A, A^W and A* strains, and the presence of slight symptoms in *F. obovata* inoculated with all three pathotype A strains and of *F. japonica* with strain C40 (pathotype A). All tested *Fortunella* spp. resulted resistant to *Xca*. Nine of the species tested were presumptively classified as non-host and only *M. ovatifoliolata* and *Eremocitrus glauca* were susceptible to all pathotypes. Bacterial population densities ranging from 10^3 to 10^6 cfu mL^{-1} were recorded in plants showing HR or no response, meanwhile, 10^7 to 10^9 cfu mL^{-1} were isolated from typical CBC lesions.

Genetic basis for basal resistance against *Xanthomonas campestris* pv. *campestris* in *Arabidopsis thaliana*

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Black Rot, caused by *Xanthomonas campestris* pv. *campestris* (*Xcc*), remains a devastating disease on Brassica crops worldwide. *Xcc* is one of few foliar bacterial pathogens that colonize its host through organs on the leaf margins, called hydathodes, which stand in direct contact with the leaf vasculature. After dissolution of the hydathode, *Xcc* colonizes the xylem vessels. Recent studies have shown that *Xcc* proliferates in the hydathode and subsequently colonizes the vasculature in a type III secretion-dependent manner, indicating that the epithem tissue in the hydathode mounts an immune response. In general, plants can perceive pathogen-associated molecular patterns (PAMPs) of pathogens by specialized Pathogen recognition receptors (PRRs). Recognition by PRRs results in PAMP-triggered immunity (PTI). Whereas PTI is well studied for mesophyll colonizing bacterial pathogens like *Pseudomonas syringae*, it remains to be shown that PTI affects xylem-colonizing bacteria like *Xcc*. Importantly, *Xcc* escapes recognition of the well studied Arabidopsis PRRs FLS2, EFR and ReMax. For other PAMP-PRR pairs recognition was demonstrated, but so far recognition has not been linked to enhanced resistance in a natural infection with *Xcc*. During infection, *Xcc*'s type III secreted effectors likely suppress PTI. In addition, it has been shown that xanthan is able to chelate divalent calcium ions from the apoplast, resulting in dampening of PTI. We have now obtained genetic evidence that PTI contributes to basal resistance against *Xcc* in a compatible interaction. Arabidopsis plants impaired in known co-receptors of a broad-range of PRRs showed enhanced susceptibility against a *Xcc* strain with its type III secreted effectors still in place except for XopAC. We are now aiming to unravel the genetic basis of this PTI.

Session 4

Disease Management – Vector Control

Chairs: Maria SAPONARI & Tamas KOVACS

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Climatic suitability for *Xylella fastidiosa* using species distribution models

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Species distribution models (SDMs) determine the relationships between sampled locations for a species and associated environmental variables, and are used to estimate the ecological requirements of the species. SDMs provide realistic scenarios to explain the influence of bioclimatic variables on the epidemiology of plant pathogens, particularly in the context of emerging plant diseases. Different modeling techniques, including regression, classification and machine learning approaches were used within an ensemble forecasting framework to quantify and map the global patterns of the potential geographic distribution of *Xylella fastidiosa*. The global distribution of *X. fastidiosa* was obtained from EFSA (EFSA Journal 14:4378, 2016). To cope with the equilibrium assumption, pseudo-absence data were generated outside the organism's ecological domain. Overall, projected potential distribution from estimated models conformed well to the current known distribution of *X. fastidiosa*. The application of SDMs to the most prevalent *X. fastidiosa* subspecies (i.e. *fastidiosa*, *pauca* and *multiplex*) will be discussed.

On preparations for timely detection of *Xylella fastidiosa* in Croatia

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Keywords: detection, PCR, control, *Olea europaea*, *Nerium oleander*, inspection

A plant pathogenic bacterium *Xylella fastidiosa* (Wells & Raju) is emerging in Europe, thus posing a growing threat for major agricultural production in certain areas especially in the Mediterranean, where Croatia is located. The bacterium is widespread in Americas on different hosts, but in 2013 it was first found in Italy in Puglia region causing extensive quick decline of olives. Still, in the EU the bacterium is quarantine pest and it is under a strict regulation. In the Republic of Croatia olive and grapevine growing represent not only deep tradition, but a prominent and strategical economic branch, even though both plants are major hosts for supervenient bacterial pathogen. In this contribution we summarize available data on preventive efforts on the national level and report approaches of preparation activities for early detection of *X. fastidiosa* potential outbreak in risky coastal areas of Croatia. Activities on the subject included improvements in laboratory detection, potential vectors survey and visual inspections focused on plants suffering from leaf scorch and symptoms of decline. Samplings were followed by laboratory testing on series of host plants. During last three consecutive years the high importance was given to preparation of experts and collaborating phytosanitary personnel, growers as well as on increasing public awareness. The review of continuous surveys performed by different participants in order to spot the possible occurrence of *X. fastidiosa*-caused diseases is presented.

Evaluating biocontrol of *Xylella fastidiosa* disease in olive with a beneficial endophyte

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Keywords: *Xylella fastidiosa*, *Paraburkholderia phytofirmans* PsJN, endophytes, biocontrol, olive

Xylella fastidiosa (*Xf*) subsp. *pauca* phylotype ST53 is responsible for a devastating disease on olive crops, in the southern area of Apulia (Italy). Despite the vast literature available on *Xf*-host relationships, scarce and barely recent efforts have been made to investigate the potential role of microbial interactions on the disease phenotype of *Xf*-infected plants. In the larger context of an ongoing characterization of the microbial community inhabiting the vascular endosphere of olive cultivars showing different susceptibility to *Xf* infection, the present study is attempting the identification of a bacterial endosymbiont that may play an antagonistic role against *Xf* disease progress. Recently, the plant growth-promoting rhizobacterium *Paraburkholderia phytofirmans* PsJN (*Pp*), known to improve plant tolerance to abiotic stresses, has been found capable of mediating resistance mechanisms against virulent bacteria. On this premise we started to evaluate the potential for using *Pp* in a biocontrol strategy, to reduce symptom severity in *Xf* ST53-infected olives. Endophyte behaviour in olive is still poorly characterized, and recent studies reported that several symbionts isolated from xylem tissues in woody crops may scarcely survive and move beyond the point of inoculation when artificially re-introduced. Here we report the successful attempt of *P. phytofirmans* strain PsJN to survive for a long-term, to reach relevant population sizes and actively move in the olive vascular system. Having established its efficient endophytic colonization in olive, further experiments are now underway to investigate if *Pp* could affect the growth of *Xf* ST53 in artificial conditions or inhibit the appearance of symptoms in olive or other susceptible indicator plants.

Susceptibility testing of cabbage breeding lines to black rot infection

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The susceptibility of twenty-four breeding lines to *Xanthomonas campestris* pv. *campestris* was evaluated. The selection of appropriate inoculation method was done before on 4 cabbage cultivars ('Cerox', 'Sintex', 'Sonja' and 'Avak'). Plants were infected by 5 inoculation assays (spraying, injection by syringe, multiple pricking, carborundum abrasion and scissor clipping method) in stage of one month old seedlings. Four different bacterial isolates of *Xcc* (WHRI 3811, 3971A, 1279A, SU) and their mixture were also evaluated for their aggressiveness on 'Cerox' and 'Sonja' cultivars. On the basis of results showing the strongest effect, breeding lines of head cabbage were inoculated by mixture of all tested isolates using multiple pricking method. The disease severity of inoculated seedlings proved high susceptibility of young plants to the *Xcc* infection. The disease incidence determined 75 and 105 days after sowing showed changes for 16 of tested lines and indicated that tolerance testing should be observed until mature stage. Two breeding lines (Kalibos and Avak1) with disease incidence lower than 20 % were chosen for repetition of resistance testing in following year. The influence of weather was also examined.

Biotic and abiotic stress extends the target range of the commercial *Bacillus amyloliquefaciens* strain MBI600 and upregulates antibiotic production and competence

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Bacillus subtilis and its related species *Bacillus amyloliquefaciens* have long been used as biological control agents (BCA). The exact mode of action in both the direct and indirect antimicrobial activity of *Bacillus* species has not been revealed, but it is thought to largely depend on the production of a vast array of secondary metabolites, mainly lipopeptides and polyketides. In this work, the effect of the commercial strain *Bacillus amyloliquefaciens* subsp. *plantarum* MBI600 (tradename Serifel®, BASF SE) and its metabolites, have been (extensively) evaluated against 13 commercially important plant pathogens, including three *Xanthomonas* species. *In vitro* assessment was based on formation of inhibition zones, inhibition of growth in broth media, growth kinetic analysis and time-lapse confocal microscopy. *In planta* evaluation against *Xanthomonas campestris* pv. *vesicatoria* was based on symptom development and population counts. *Xanthomonas* species were found sensitive. BCA endospores grew and formed biofilms in the plant phyllosphere, maintaining for several days high population levels and thus offering protection in tomato plants. Plant exudates stimulated BCA antibiotic production, chemotaxis and competence; a similar effect was induced by plant pathogens. In this work, the induction of plant defense by a BCA was evaluated for the first time using commercial formulation of different dosage, by means of microarray analysis and qPCR. Finally, it was shown that the palette and quantity of antibiotics produce by BCA increased under abiotic stress, resulting in the expansion of plant pathogenic bacteria target range.

Potential of random peptide mixtures as crop protection agents against plant diseases caused by *Xanthomonas* and other plant pathogenic bacteria

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Keywords: Antimicrobial peptides, *Xanthomonas perforans*, *Xanthomonas campestris* pv. *campestris*

Many types of crops are severely affected by at least one important bacterial disease. Chemical control of bacterial plant diseases in the field vastly relies on copper-based bactericides, yet with limited efficacy. In the present study, we explored the potential of two random peptide mixture (RPM) models as novel crop protection agents. These unique peptide mixtures consist of random combination of L-phenylalanine and L/D-lysine (FK-20 and FdK-20, respectively) along the 20-mer chain length of the peptides. Both RPMs displayed powerful bacteriostatic and bactericidal activities towards strains belonging to several plant pathogenic bacterial genera including *Xanthomonas*, *Clavibacter* and *Pseudomonas. In planta* studies in the greenhouse revealed that RPMs significantly reduced disease severity of tomato and kohlrabi plants infected with *Xanthomonas perforans* and *Xanthomonas campestris* pv. *campestris*, respectively. Moreover, RPM effects on reduction of disease severity were similar to those exerted by the commercial, copper-based bactericide Kocide 2000, that was applied at 12-fold concentration of the active compound relative to the RPM treatments. Importantly, the two tested RPM compounds had no toxic effect on survival of bees and Caco-2 mammalian cells. The present study demonstrates the potential of these innovative RPMs to serve as crop protection agents against crop diseases caused by *Xanthomonas* species and other phytopathogenic bacteria.

Antibacterial effect of selected nanoparticles as revealed by doubling time of treated *Xanthomonas campestris* pv. *campestris* cultures

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Keywords: Nanoparticles, *Xanthomonas campestris*, doubling time, antibacterial effect

Besides many possibilities of applications of nanoparticles in the field of medicine, diagnostics, molecular biology, bioorganic chemistry or remediation of environment, there is also a potential of employment of nanoparticles as a tool for elimination and control of bacteria invading plant tissue. In this experiment an antibacterial activity of selected nanoparticles based on silver, gold and bimetallic silver/copper was tested in vitro on bacteria cultures of *Xanthomonas campestris* pv. *campestris* (*Xcc*) (strain 1279a). The strongest inhibitory effect represented by doubling time of treated cultures was measured in the presence of the smallest silver nanoparticles (9 nm) at the highest concentration (5 ppm). Based on these results the eradicating effect of silver nanoparticles against *Xanthomonas campestris* pv. *campestris* on infected seeds of cabbage was tested using real-time PCR.

POSTERS

Investigation on the susceptibility of tomato and pepper cultivars to *Xanthomonas euvesicatoria* strains causal agent of bacterial spot disease

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Keywords: *Xanthomonas euvesicatoria*, bacterial spot of tomato and pepper, diagnosis, cultivar susceptibilty

Bacterial spot of tomato and pepper caused by Xanthomonad species has been significant problem for tomato and pepper cultivation in Turkey. It has been caused economical losses due to affected the yield and quality of fruit. It was known unique Xanthomonad species which was named *Xanthomonas campestris* pv. *vesicatoria* as causal agent of bacterial spot on tomato and pepper up to 1990s. According to the recent developments on bacterial taxanomy, there are four *Xanthomonas* species as causal agent of bacterial spot on tomato and pepper: *X. vesicatoria* (*Xv*), *X. euvesicatoria* (*Xe*), *X. perforans* (*Xp*), and *X. gardneri* (*Xg*). The main causal agent of bacterial spot on tomato and pepper plants in Turkey has been identified as *X. euvesicatoria* (*Xe*) in our work by biochemical and molecular techniques. The management methods for bacterial spot are including sanitation, application of chemicals, cultural practices and use of resistant cultivars to the disease. Copper-based chemicals have been commonly used for controlling the disease. However, copper–resistant strains of the pathogen have been reported in Turkey. Copper-resistant strains of Xanthomonads have been decreased the efficacy of chemical control especially in field conditions. Therefore, the most practical and economical method for bacterial spot management has been considered as use of resistant cultivar/s against pathogen. The aim of this study was to investigate the susceptibility of commonly cultivated tomato and pepper cultivars to different strains of *Xe*. All of the tested tomato and pepper cultivars were determined as susceptible to bacterial spot disease caused by *Xe* strains, but among cultivars were detected differences in terms of disease severity. On tomato, the lowest disease severity and the highest disease severity was detected in Ferman F1 and TMOSO38 F1, respectively. On pepper, the lowest disease severity and the highest disease severity was determined in Yıldız F1 and King Bell F1, respectively.

Design and testing of multiplex-PCR primers for detection of bacterial spot of tomato

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Keywords: *Xanthomonas*, tomato, pepper, bacterial spot, multiplex PCR

This study presents development of multiplex-PCR assay for specific detection of plant pathogenic bacteria of *Xanthomonas* genus causing bacterial spot of tomato and pepper. PCR primers for differentiation of *X. euvesicatoria, X. vesicatoria* and *X. gardneri* were developed based on the DNA sequences of bacterial type strains. Primer pairs were designed and subsequently thoroughly tested and optimized for parallel detection of the bacteria. Specificity of the primers was tested on a large complex of bacterial strains pathogenic to tomato, pepper and related crops. Following the described protocol *X. euvesicatoria*, *X. vesicatoria* and *X. gardneri* can be quickly and reliably identified in a single multiplex-PCR assay.

Epidemiology of bacterial diseases caused by *Xanthomonas* spp. in Bulgaria

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Bacterial diseases in Bulgaria have always been of interest because of their significance, especially when rainy periods attend the plant's vegetation. On the other side, a tendency of increasing import of seeds and plantlets has enhanced the threat in that direction. In order to study such a revivification of bacterial diseases, plant material with symptoms suspected to be of bacterial origin have been subject of large-scale field, nursery and green-house surveys within a 5-year period (2013/17). The dominant bacterial types have been isolated and subjected to polyphasic laboratory and plant testing (colony type, HR, pathogenicity, PCR). As a result, representative collection of more than 50 pathogenic *Xanthomonas*-resembling strains was also created. These representatives of the genera were isolated from about 20 different host (cereals, horticultural and ornamental) species that were cultivated in 7 different districts of the country. The final identification of the whole group is under way. Meanwhile, the working collection is expanding, the representative strains being described and stored in the public service collection of LMG (Belgium). Undoubtedly, the action at national level will allow identifying new and emerging threats for the country and at the same time will provide more valuable information on the spread and diversity of pathogenic xanthomonads in different plant species and climate regions within Europe.

Is *Xylella fastidiosa* a risk for Belgium? Ongoing investigations on potential hosts and insect vectors

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Based on the previous EFSA opinion on the risks to plant health posed by *Xylella fastidiosa* in the EU territory, the risk of *X. fastidiosa* for a country like Belgium is evaluated. Based on a first screen of the Xylella host database, potential hosts plants for *X. fastidiosa* (EFSA, 2015), like *Prunus domestica* cv Opal, *Quercus petraea* and *Salix alba* have been selected and inoculated with different strains of *X. fastidiosa* subsp. *fastidiosa* and *multiplex* as well as the CoDiRo strain, to evaluate their susceptibility. The potential for the bacteria acquisition through artificial diets and artificially infected plants is also studied for potential insect vectors like *Cercopis vulnerata*, *Cicadella viridis*, *Philaenus spumarius*, *Aphrophora alni*, *Aphrophora salicina,* with the help of a GFP-labelled *X. fastidiosa (Newman et al., 2003).* With the view of supporting field surveys, a PCR detection scheme combining the detection of both the bacteria and potential insect vectors has also been developed and is compared to existing detection methodologies. The idea of targeting carefully chosen insect species, with the view of using the insect spy strategy for the early detection of the bacteria will be discussed.

Arming and disarming against pathogens: Proteasomal protein turnover during plant defenses

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The plant immune system comprises a dual defense strategy against pathogens, ensuring efficient and fast control of the infection. *Xanthomonas* suppresses both plant defense strategies, the PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) by the injection of TypeIII effector proteins (T3Es) into the host cell. Approximately 40 T3Es have been identified in *Xanthomonas* so far. Although T3Es are assumed to contribute to virulence of *Xanthomonas*, host cellular targets and biochemical activities for many effectors remain unknown. The plant proteasome is a crucial component of plant immunity and has earlier been demonstrated to be directly targeted by pathogenic effectors. Our study identified a pepper E3-ligase (BRGx) as well as a WRKY transcriptional regulator as targets of the *Xanthomonas* effector XopS. Both, BRGx and WKY40 are constantly fed into proteasomal protein turnover. While WRKY40 presumably functions as transcriptional repressor of defense genes, BRGx mediates WRKY40 protein degradation. XopS interferes with this process by mimicking E3 ligase activity and thereby ubiquitinating and stabilizing WRKY40. The mechanistic details of ubiquitination-based protein stabilization in this process remain to be elucidated.

Improving the typing of *Xylella fastidiosa* directly from plant material

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Keywords: MLSA, MLST

Detection of *Xylella fastidiosa* directly from plant material is a common practice as a screening test for epidemiosurveillance purposes. MultiLocus Sequence Analysis and Typing (MLSA/MLST) methods are amongst the most widely used genotyping methods for assessing the global epidemiology of various plant pathogenic bacteria including *X. fastidiosa*. MLSA is used to assign strains to one of the known *X. fastidiosa* subspecies and MLST for typing strains and make inference of their origin. Both methods are traditionally used on isolated strains. Nonetheless, because it is time-consuming and sometimes difficult to obtain isolates of *X. fastidiosa* from plant samples as a consequence of an intrinsically poor isolation rate and the fastidious nature of the pathogen, being able to type *X. fastidiosa* in plant samples is a priority for epidemiological purposes. Application of the initial protocol on DNA extracted from plant material proved limited efficiency. The aim of our study was to test the impact of various modifications of the protocol in order to improve the efficiencies of DNA extraction and target amplification rate. We also tested the interest of reduced typing schemes. The direct identification of *X. fastidiosa* infecting plant material face however some limitations that will be discussed.

Insights into *Citrus limon* nonhost and host resistance against *Xanthomonas* spp.

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Citrus is an economically important fruit crop that is severely afflicted by Asiatic citrus canker, a disease caused by the bacterial *Xanthomonas citri* subsp. *citri* (*X. citri*). There is great need for new and sustainable strategies to manage citrus canker disease. Strong evidence in several pathosystems indicates that nonhost resistance is more durable and robust compared with host resistance. In order to identify novel resistance mechanisms and genes with a role in canker resistance, we have characterized at the histological and molecular level the main differences between the defence response in *Citrus limon* to the non-adapted pathogen *X. campestris* pv. *campestris* (*Xcc*) and to the host pathogen *X. fuscans* subsp. *aurantifolii* strain C (*Xfa* C). *Xfa* C develops necrotic hypersensitive response (HR) in *C. limon*. Our results indicate that *Xcc* induces type I nonhost resistance in *C. limon* without necrotic hypertensive response. Both nonhost and host defense responses against *Xcc* and *Xfa* C, respectively, interfere with biofilm development on *C. limon* leaves. However, differential patterns of reactive oxygen species, accumulation of phenolic secondary metabolites and genes related to SA-defense signalling pathway are observed between nonhost and host resistance. Furthermore, both defense responses protect *C. limon* plants from disease upon subsequent challenges by pathogenic *X. citri*. This knowledge will rationally exploit the plant immune system as a biotechnological approach to manage the disease.

Differentiation and control of *Xanthomonas* spp. pepper and tomato pathogens in Serbia

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Key words: *Xanthomonas euvesicatoria*, *X. vesicatoria*, races, bacteriophages, biocontrol

Xanthomonas spp. complex regularly affects pepper and tomato grown in Serbia. Routine application of copper compounds failed to prevent occurrence of leaf spot, blight and fruit scab, especially in weather conditions favoring the infection. Lack of commercially available pepper and tomato cultivars resistant to the pathogen, as well as using seed of questionable quality, make the disease control even more difficult. So far, four races (P1, P3, P7, P8) of *Xanthomonas euvesicatoria*, the pepper pathogen, and one tomato race (T2) of *X. vesicatoria*, have been recorded in Serbia. Integrated disease management practices, including biocontrol agents were studied in order to develop an efficient and sustainable disease control strategy. Bacteriophage strains specific to *X. euvesicatoria* were isolated and studied. The results showed that foliar application of host-specific phages can effectively reduce severity of pepper bacterial spot compared to the untreated control. In order to integrate different protection methods in control of the disease, we studied efficacy of biocontrol agents (bacteriophages and *Bacillus subtilis*), SAR inducer (ASM), antibiotics (streptomycin), as well as copper based compounds. The most efficient treatment was the integration of ASM, copper hydroxide and bacteriophages. This combination may be a new effective tool for pepper growers to manage pepper bacterial spot.

Multilocus sequence analysis of xanthomonads associated with tomato, pepper and alfalfa in Iran identifies novel population structure of *X. euvesicatoria* pv. *perforans*

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During the last years, three quarantine bacterial diseases *i.e.* bacterial spot of tomato and pepper, and bacterial leaf spot of alfalfa, caused by pathovars of *Xanthomonas euvesicatoria* (a.k.a. *Xanthomonas perforans*, *X. euvesicatoria*, and *Xanthomonas alfalfae* subsp. *alfalfae,* respectively), have been reported in Iran. We have performed a multilocus sequence analysis (MLSA) to decipher the phylogenetic position of xanthomonads associated with tomato, pepper, and alfalfa in Iran. MLSA on five housekeeping genes (*i.e. fusA*, *gapA*, *gltA*, *lacF*, and *lepA*) showed that *Xanthomonas* strains isolated from tomato in Iran were clustered in a monophyletic group with 10 nucleotides differences in the *lepA* gene from the worldwide population of this pathovar. These strains were phylogenetically closer to *X. euvesicatoria* pv. *euvesicatoria* than to *X. euvesicatoria* pv. *perforans*. A significant recombination (which occurred on *lepA* gene) has been detected in the strains isolated from tomato in Iran. Furthermore, *X. euvesicatoria* pv. *euvesicatoria* isolated from pepper in Iran, were clustered in a group containing strains isolated in Nigeria, which differed in one nucleotide in *lepA* from the worldwide population of the species. Xanthomonads isolated from alfalfa in Iran were clustered with the type strain of *X. euvesicatoria* pv. *alfalfae*, while five additional strains isolated from poinsettia were also clustered in this group. Altogether, the results of this study revealed that an atypical but non-clonal population of *X. euvesicatoria* pv. *perforans* is emerging in Iran, which needs further molecular studies to find the geographical origin of the strains.

Phage and aldehyde work in synergy to control *Xanthomonas* infection

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Keywords: *Xanthomonas*, black rot, bacteriophage, aldehyde, infection

Xanthomonas campestris pv. *campestris* (*Xcc*) is a Gram-negative bacterium that causes black rot, one of the most important diseases of vegetable brassica crops worldwide. The use of bacteriophages for the control of vegetable diseases is a sector of growing interest, providing more advantages than the use of chemicals in agriculture. In this study, we isolated and characterized a lytic bacteriophage from the soil, capable of reducing *Xcc* infection. We evaluated the antimicrobial activity of the phage, and its possible direct administration to the plant xylem. Further, tests were performed both in *in vivo* and *in vitro* experiments to assess the activity of the bacteriophage in association with several anti-biofilm molecules, such as a long-chain fatty aldehyde and its analogs, that differed in the length of the aliphatic chain, obtained from an Antarctic *Pseudoalteromonas haloplanktis*. We demonstrated that the synergism between the bacteriophage and anti-biofilm molecules could be the most effective way to breakdown the biofilm and control *Xanthomonas* infection.

Phylogeography and population structure of *Xanthomonas fragariae* to identify sources and pathways of this bacterial phytopathogen through plant material trade

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Keywords: CRISPR, VNTR, MLVA, *Xanthomonas fragariae*, strawberry

Angular leaf spots are caused by *Xanthomonas fragariae* with particular severe effects under protected cultivation with high-density plots aided by high humidity and sprinkler irrigation systems. *X. fragariae* was first observed in the USA in 1960, in Europe in 1970 and then spread worldwide in strawberry growing regions, causing yield loss of harvested fruits. Its quick spread is thought to be due to importation of plant material through trade and more generally of human activities. There is a real need for reliable methods accurately discriminating between strains for crop surveillance, outbreak investigation and establishing disease control strategies. As relatively low diversity was observed among 59 *X. fragariae* strains of various geographic and time origins, the discrimination power of several molecular markers was determined. Among these markers for source tracking purpose, variable number of tandem repeats (55 VNTRs) were used as an efficient genotyping method, where numbers of repeats acted as molecular clocks with sufficient resolution to discriminate strains. In addition, clustered regularly interspaced short palindromic repeats (2 CRISPRs) were useful markers, also used in epidemiology and host-bacteria surveys, bringing complementary information for genetic diversity characterization and direction of evolution. This study highlighted the flux of evolution of *X. fragariae* strains and two main origins of evolution that could be responsible for the world-wide invasion of *X. fragariae*. An understanding on population structures of the quarantine pathogen *X. fragariae* and how the disease can emerge and spread over a given geographical region are trivial for bacterial monitoring.

Study of *Xanthomonas arboricola* pv. *corylina* population occuring on hazelnut in Serbia

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Keywords: *Xanthomonas arboricola* pv. *corylina* (*Xac*), hazelnut, bacterial blight, identification, characterization

Xanthomonas arboricola pv. *corylina*, a causal agent of hazelnut bacterial blight, may cause significant economic losses, particularly in young hazelnut plantations and nurseries in weather conditions favoring infection. In Serbia, the pathogen has been occasionally detected mainly on plants over the country, as well as on latently infected imported planting material. The occurrence of the disease was monitored several years and the pathogen was isolated from samples collected in different locations. To gain an insight into population structure of *Xac* strains occuring in Serbia, forty seven strains were characterized. Based on the phenotypic features and Biolog test, the strains were assigned to *Xanthomonas* spp. The ability of strains to metabolize quinate and PCR analysis using XarbQF/XarbQR primers supported their identification to the species level. Forty four tested strains gave positive amplification signal with primers specific for *X. a.* pv. *pruni*. Pathogenicity assays, performed by bud inoculation of one-year-old susceptible hazelnut plants in a greenhouse, confirmed that the strains belong to hazelnut pathogens. Molecular characterization using DNA fingerprinting techniques (rep-PCR and PFGE) indicated significant genetic diversity and heterogeneity of the Xac strains present in Serbia. Sequencing of 16S rDNA revealed 98-100% sequence similarity with different *Xanthomonas* spp., providing identification only to the genus level. The *rpoD* sequence analysis indicated that the strains belong to a homogenous genetic group within *Xanthomonas arboricola* pv. *corylina*, distinct from other strains of the same species, confirming identity at pathovar level.

Functional characterization of the *Xanthomonas campestris* pv. *vesicatoria* type III effector protein XopS

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Xanthomonas outer protein S (XopS) is a so-called typeIII effector protein (T3E), injected by *Xanthomonas campestris pv. vesicatoria,* the main pathogenic agent responsible for the bacterial leaf spot disease in agronomical relevant crop plants. Since XopS is responsible for disease symptom development in pepper, its functional characterization is crucial for the understanding of how *Xanthomonas* is able to manipulate plant defense strategies by introducing XopS to the host cell.

We could demonstrate the interaction between XopS and the transcription factor WRKY40, as well as the E3 ligase BRGx in *Nicotiana benthamiana.* BRGx seems to ubiquitinate WRKY40 upon pathogen attack, leading to the proteasomal degradation of the defense gene repressor. XopS itself seems to mimic an E3 ubiquitin ligase activity. XopS is assumed to ubiquitinate and thereby stabilize the transcription factor WRKY40. In our ongoing work we are identifying the WRKY40 ubiquitination sites and are aiming to discover which type of K-linkages are associated either with its turnover or stabilization. Since the structural requirements for XopS to function as E3 ligase are unknown, we are currently investigating the different predicted structural domains for their enzymatic activity.

Bacterial wilt of wallflowers (*Erysimum* spp.) is caused by known and novel races of *Xanthomonas campestris* pv. *campestris*

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Keywords: *Xanthomonas*, wallflowers, races

Bacterial wilt or blight of wallflowers was first reported in the UK in 1970 and has been a significant problem for commercial growers in recent years. Symptoms can be vague and variable: affected leaves often have one-sided chlorosis before dying, whole plants may be stunted, readily shed their lower leaves, and are short-lived. Several papers have reported that *Xanthomonas campestris* strains from wallflowers (*Erysimum* spp.) are not pathogenic to *Brassica* spp. and may represent a distinct pathovar. In contrast, isolates obtained during nursery surveys and from clinic samples were found to be pathogenic on *Brassica oleracea,* and subsequently tested for pathogenicity on the brassica differential series. Most isolates displayed a pattern of reactions consistent with Race 6, and a smaller number were consistent with races 5 and 9. However, one group of isolates displayed a pattern of reactions which appear to represent a novel race.

Antibacterial activity of plant extracts against *Xanthomonas* pathogens of tomato

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Keywords: plant extracts, tomato, antibacterial activity, *Xanthomonas vesicatoria, Xanthomonas gardneri*

Tomato plants of all ages are susceptible to pathogens from genus *Xanthomonas* sp. Control in Bulgaria is extensively based mainly on copper-containing chemicals and is often unsatisfactory. We prepared extracts from different plants and tested them for antibacterial activity *in vitro* against referent isolates.

Fresh plant aerial parts were oven-dried or freezed before extraction. Methanol and *n*-hexane were used as solvents. Extractions were prepared in Soxhlet extractor at 80°C/4h. Methanol and *n*-hexane were recovered in vacuum evaporator. The fractions were diluted in water (%, w/v) with dimethylsulfoxide as dilution agent for some extracts. The *in vitro* test was completed by the agar diffusion method in triplicate with 50 µl of each substance. The antimicrobial activity was assessed by measuring the diameter of the inhibition zone.

A total of 25 plants and 47 methanol and *n*-hexane extracts were tested against the xanthomonads of tomato. Extracts from eight plants have some antibacterial effect against the pathogens. Methanol extract from *Chelidonium majus* and *Chaenomeles* sp. have biggest potential for control of *Xanthomonas* of tomato. *Hylotelephium spectabile* has potential for control but higher concentrations need to be tested.

Innovative plant protection technologies for quarantine pathogens of *Xanthomonadaceae* family utilizing tools of optoacoustics and molecular biology

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Keywords: Innovative disease diagnostics, Microbial diagnostics, plant microbiology, bacteriology, plant diseases

In Crete, there is a particular focus on quarantine plant pathogens and the last year there is an implementing activation plan for the potential outbreak of *Xylella fastidiosa* (*Xf*). The Laboratories at UASC/TEIC are fully equipped for the detection and molecular identification of all plant pathogenic bacteria. We are also responsible for all certified survey controls done in the whole region of Crete, appointed by the Greek Ministry of Rural Development and Food. During 2016, 315 samples analyses were carried out of olive trees and other potential hosts of the bacterium from the four Regional Units of Crete. Recently, the INNOVA-PROTECT project, has been submitted and is expected to involve, three academic/research institutions that will launch cooperation with private companies in Greece to set up a multidisciplinary research team for the establishment of a system regarding the direct and reliable diagnosis of plant pathogenic bacteria of the *Xanthomonadaceae* family. Due to the severity of the plant diseases caused by *Xanthomonadaceae*, and for the protection of the plant capital of Greece, it is imperative to find a system for the early and reliable diagnosis of these pathogens. One of the main ways to spread a pathogen (a new disease) is to import contaminated plant reproductive material (e.g. grafted plant material, seeds, etc.). This is particularly important, considering one of the most significant pathogens of this family, *Xf*. It has already been settled in neighboring Italy

and to other European countries, while there is an increased risk to be transported in Greece, and in Crete as olive trees and grapes cover more than 70% of the agricultural economy. In this project, we initially propose the construction of a novel optoacoustic detection system for rapid diagnosis of plant pathogens that can be installed at the country's entry points (e.g. customs) and will be able to distinguish whether the migrant propagating material bears inoculum from a quarantine pathogen. Moreover, we have developed a metabolically engineered *E. coli* system that is able to produce plant phenolics that have been found to inhibit growth of several bacterial species including members of *Xanthomonadaceae* family, thus providing new possible therapeutic tools environmentally safe. Additionally, we recommend the development of micro-arrays for the quick and direct detection and identification of plant pathogenic *Xanthomonadaceae*, using plant tissue fluids. At the same time, we propose the study of specific herbal preparations regarding the control.

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Integrating science on *Xanthomonadaceae* for integrated plant disease management in Europe

cost
EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

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Participating Countries
BA, BE, BG, EE, ES, CH, CZ, DE, FR, GB, GR, HR, HU, IL, IT, MK, NL, NO, PL, PT, RS, SE, SI, TR

Challenge

Present, emerging or re-emerging plant diseases due to infection by of the genera *Xanthomonas* and *Xylella* are continually challenging food security and cause significant losses to the EU economy each year, thus demanding for concerted R&D actions at the international level, which will be supported by the COST Action networking instruments.


Olive trees cut down because of *Xylella*. Photo credit: PanareoFotografia via Thinkstock

Working Groups

WG1 – Diagnostics & Diversity
WG2 – Pathogen Biology
WG3 – Resistance & Defence
WG4 – Disease Management

Objectives

- ✓ Develop, implement, compare and standardize methods of pathogen detection
- ✓ Estimate the risk of epidemics and outbreaks
- ✓ Develop, distribute and valorize bioinformatics tools for data analysis
- ✓ Identify key bacterial factors in the microbe-eukaryote interaction at different steps of the infection/dissemination cycle
- ✓ Identify elicitors of plant defense responses as targets for resistance breeding
- ✓ Discover novel resistance traits
- ✓ Generate durably resistant crop cultivars
- ✓ Evaluate and establish disease control measures
- ✓ Evaluate and compare approaches to eliminate or reduce vector populations

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