



# 3<sup>rd</sup> International Workshop on Interactions between crop plants and human pathogens

Organized by

**CA COST Action CA16110** 

**Control of Human Pathogenic Micro-organisms in Plant Production Systems** 

**WP1** Ecology of HPMO in plants and in environments relevant for plant production

Food-borne disease outbreaks resulting from consumption of plant-derived fresh produce have been reported worldwide such as from spinach in the USA, from mung bean sprouts in Japan and most recently also in Europe from fenugreek sprouts (Hamburg, 2011). It is clear that particular groups of human pathogenic micro-organisms (HPMO) can find their ecological niches in plant production systems. Contamination routes of HPMO to plants are poorly understood. Basic resources for agroproduction, such as soils, water and fertilizers can play a role in contamination of plants, but microorganisms taxonomical closely related with HPMO are also present in plant microbiomes. HPMO must be considered as integral components of the plant microbiome and it is the intention of HUPLANTcontrol to investigate the potential negative aspects of plant microbiomes on human health and to integrate novel scientific insight into sanitary measures and agricultural management practices. The HUPLANTcontrol network consists of five working groups: 1) on the ecology of HPMO in plants, 2) on taxonomical identification of HPMO from plants, 3) on characterization of the potential human-threatening nature of HPMOs, 4) on sanitary and agricultural management procedures to control HPMO in plant production facilities and 5) on dissemination of achieved knowledge via connections between science groups and relevant stakeholders from agriculture, industry and public health authorities. The Action integrates molecular biology, bio-informatics, microbiology, ecology, agronomy, veterinary and clinical sciences and places a strong focus on primary plant production, in principle covering all micro-organisms posing potential threats to humans.

#### Organizers

#### **Adam Schikora**

Julius Kühn-Institut Federal Research Centre for Cultivated Plants (JKI), Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany

#### Katarzyna Hrynkiewicz

Department of Microbiology, Nicolaus Copernicus University, Toruń, Poland

Local Organizer

#### **Rita Grosch**

Leibniz Institute of Vegetable and Ornamental Crops Großbeeren, Germany

Info & Registration: Adam Schikora (adam.schikora@julius-kuehn.de)

Organization: Rita Grosch (grosch@igzev.de)





### **General Information**

**Dates** 12. - 13. March 2018 and 14.03.2018 (MC Core)

Venue Humboldt-Universität zu Berlin, Lebenswissenschaftliche Fakultät

Lentzeallee 55-57, 14195 Berlin

**Room** 113 and 114

**Accommodation** Novum Hotel Ravenna Grunewaldstraße 8-9, Steglitz, 12165, Berlin

Hotel Steglitz, Albrechtstraße 2, 12165 Berlin

Please book the hotel yourself!

**Lunch** Will be provided on 12 and 13. March during the lunch pauses

**Stakeholder meeting** Tuesday 13.03.2018 (14:30 – 17:00)

For all patricians of the workshop

Social Program Guided tour in Berlin Botanical Garden (Group in De and EN)

Tuesday 13.03.2018, 17:30 - 18:00





### Time table:

Monday 12.03.2018		Tuesday 13.03.2018		Wednesday 14.03.2018	
9:00 – 9:15	Registration & Welcome Rita Grosch: Overview of the COST-Action	9:00	Registration	9:00	Registration
9:15 – 10:00	Invited talk  Maria Brandl  Produce safety from farm  to table: Rare  opportunities for  opportunists at various  scales	9:00 – 11:30	Session III Chair: Kornelia Smalla Mobile elements: antibiotics resistance, epigenetics, phages, mechanisms of transmission.	9:00 – 12:00	MC Core Meeting (for CA16110 MC members only)
10:00 - 12:30	Session I Chair: Gabriele Berg Microbiome: definitions, "opportunistic" pathogens and food issues, presence of human pathogens in native microbiome.	11:30 - 13:00	Session IV  Chair: Adam Schikora  Internalization of human pathogens in plant tissues.		
12:30 - 13:00	Lunch	13:00 - 14:30	Lunch	12:00 - 13:30	Lunch pause
13:00 - 14:00	EFSA Presentation Maria Teresa da Silva Felicio Risk posed by pathogens in food of non-animal origin: EU outbreak data analysis and risk ranking (2011-2015)	14:30 - 17:00	Stakeholder meeting AG "Human Pathogens on Crop Plants" meeting with COST members and EFSA (for all participants of the workshop)	14:00	Departure
14:00 - 15:00	CEBAS Presentation  Ana Allende Prieto  Risk posed by pathogens in food of non-animal origin: what is going on lately at the CEBAS-CSIC?				
15:30 - 18:00	Session II Chair: Katarzyna Hrynkiewicz Effect of plant physiology, plant responses, growth stage, growth conditions and environment on the persistence.	17:30 - 18:30	Guided visit to Botanical Garden Berlin		
19:00	Conference Dinner Restaurant Englers	19:00	Free evening	1	





### **Invited speakers:**

#### Maria Brandl (USA)

United States Department of Agriculture, Agricultural Research Service, Produce Safety and Microbiology Research: Albany, CA, USA

Title: Produce safety from farm to table: Rare opportunities for opportunists at various scales

#### Maria Teresa da Silva Felicio (Portugal)

European Food Safety Authority (EFSA)

Title: Risk posed by pathogens in food of non-animal origin: EU outbreak data analysis and risk ranking (2011-2015)

#### Ana Allende (Spain)

CEBAS-CSIC Centro de Edafología y Biología Aplicada del Segura

Titel: Risk posed by pathogens in food of non-animal origin: what is going on lately at the CEBAS-CSIC?

### **Sessions:**

1.Microbiome: definitions, "opportunistic" pathogens and food issues, presence of human pathogens in native microbiome.

**Chair: Gabriele Berg** 

2. Effect of plant physiology, plant responses, growth stage, growth conditions and environment on the persistence.

**Chair: Katarzyna Hrynkiewicz** 

3.Internalization of human pathogens in plant tissues.

Chair: Adam Schikora

4. Mobile elements: antibiotics resistance, epigenetics, phages, mechanisms of transmission.

Chair: Kornelia Smalla





### **Stakeholder Meeting**

Tuesday 13.03.2018

Detailed program of stakeholder meeting

14:30 – 17:00	Stakeholder meeting
17.50 - 17.00	Working Group Human pathogen meeting with EFSA, BMEL, BLE and COST
	members
	Chair Mieke Uyttendaele
	(Uni. Gent, chair COST 16110 WG 5 - Dissemination)
	Co-chair Adam Schikora
	(Julius Kuehn institute, local organizer)
14.30-14.50h	Mieke Uyttendaele, COST HUPlantControl
	'Facilitating communication on when plant becomes food and microbes
	become pathogens"
14.50-15.10h	Sven Jechalke, JLU Giessen
	Uptake of Salmonella and E. coil into crop plants; the <i>plantinfect</i> consortium
15.10-15.30h	Charles Franz, MRI Kiel
	Human pathogens and antibiotic-resistant enterobacteria in fresh produce
15.30-15.45h	Mieke Uyttendaele
	Introduction of topics for discussion
15.45-17.00h	Open discussion
	(in English/German)

### **Topics**

- 1. The plant microbiome: source of human pathogenic bacteria or a bacterial community to provide resilience towards human pathogens' persistence and proliferation?
- 2. Dealing with (opportunistic) human pathogens in fresh produce: hazard identification versus risk assessment.
- 3. Safe or unsafe: how, when, why, to whom can or should scientific networks communicate results and outcome without raising concerns on food safety?
- 4. Trends and changes in plant species or cultivars and plant production systems: opportunities for better control or new threats emerging?
- 5. Usefulness and design of sampling plans and test methods, in research studies or as a control measure in the food supply chain (pre- and postharvest) to assess safety of fresh produce.





### MC core meeting

only for members of the MC

Wednesday 14.03.2018 (9:00 – 12:00)

Humboldt-Universität zu Berlin, Lebenswissenschaftliche Fakultät Lentzeallee 55-57, 14195 Berlin

Seminar Room

### **Topics**

→ For detailed information, please refer to email from 19.01.2018 sent by Leo van Overbeek





### **Session schedule**

# Day 1

### Monday 12.03.2018

	Widilday 12.03.2010
9:00 – 9:15	Registration & Welcome
	Rita Grosch
	Overview of the COST-Action
9:15 – 10:00	Invited talk
	Maria Brandl
	Produce safety from farm to table: Rare opportunities for opportunists at various scales
coffee	
10:15 – 12:30	Session I
	Microbiome: definitions, "opportunistic" pathogens and food issues, presence of human pathogens in native microbiome.
	Chair: Gabriele Berg
10:15 – 10:45	Gabriele Berg
	Plants as a reservoir for emerging multi-resistant human pathogens
10:45 – 11:15	Nicola Holden
	The risk to consumers by internalised human pathogens in vegetables
11:15 – 11:45	Charles Franz
11:45 – 12:00	Microbiota at different stages of industrial processing of ready to eat salad  Lofti Fki
11:45 – 12:00	Human pathogenic microorganisms in in vitro plant tissue cultures: the state of the art
12:00 – 12:15	Silke Ruppel
12.00	The phyllosphere of <i>Lepidium sativum</i> – a habitat of human probiotic or pathogenic bacteria?
12:15 - 12:30	Matthias Becker
	To be or not to be a pathogen: Differentiate true virulence factors of bacteria from false positives
12:30 - 13:00	Lunch
13:00 - 13:45	EFSA Presentation
	Maria Teresa da Silva Felicio
	Risk posed by pathogens in food of non-animal origin: EU outbreak data analysis and risk ranking (2011-2015)
14:00 – 14:45	CEBAS Presentation
14.00 14.45	Ana Allende
	Risk posed by pathogens in food of non-animal origin: what is going on lately at the CEBAS-CSIC?
coffee	
15:55 – 18:00	Session II
	Effect of plant physiology, plant responses, growth stage, growth conditions and environment on the
	persistence.
45.55 46.45	Chair: Katarzyna Hrynkiewicz
15:55 – 16:15	Katarzyna Hrynkiewicz  How do environmental factors affect infection of plants by the Human Pathogenic Microorganisms (HPMO)?
16:15 – 16:30	Adriano Sofo
10.13 10.30	Effect of irrigation with urban wastewater and sustainable soil management on the presence and persistence
	of potential HPMOs in olive trees
16:30 - 16.45	Barbara Reinhold-Hurek
	To be or not to be inside: A complex role of bacterial factors for endo- or epiphytic colonization
16:45 – 17:00	Azhar Zarkani
	The impact of Salmonella type III secretion effectors on plant defense-related genes and the proliferation on
17:00 _ 17:15	plant surfaces
17:00 – 17:15	Julia Aguilera  Dual expression of the Salmonella effector SrfJ in mammalian cells and plants
17:15 – 17:30	llana Fratty
	Cellulases of Salmonella Typhimurium and their role in Salmonella-plants interactions
19:00	Conference Dinner - Restaurant Englers





# Day 2

#### Tuesday 13.03.2018

9:00	Registration		
9:00 – 11:30	Session III Mobile elements: antibiotics resistance, epigenetics, phages, mechanisms of transmission Chair: Kornelia Smalla		
9:00 – 9:45	Kornelia Smalla		
9:45 – 10:15	Tracing the transferable resistome in bacteria of the agro-ecosystem  Tomislav Cernava		
10:15 – 10:45	Assessment of antibiotic resistances in the arugula microbiome  Robert Czajkowski  Lytic bacteriophages in agricultural environment - a (new or rediscovered?) way to control bacterial		
10:45 – 11:00	infections in crop plants  Alex Samusev  Alternative view on the LPS importance in the Salmonella-plants interactions		
11:00 – 11:15	Gregor Fiedler Characteristics of ESBL-producing <i>Enterobacteria</i> isolated from sprouts		
	coffee		
11:30 – 13:00	Session IV Internalization of human pathogens in plant tissues.		
11:30 – 12:00	Chair: Adam Schikora Adam Schikora		
11.30 – 12.00	Colonization patterns on crop plants: same-same but different?		
12:00 - 12:30	Sven Jechalke		
	Factors influencing the survival of Salmonella enterica in soil and the colonization of crop plant		
12:30 - 12:45	Jasper Schierstaedt		
	High diversity of soil microbiome reduces survival of Salmonella in the phytosphere		
12:45 – 13:00	Laura Elpers		
	Molecular analysis of the impact of adhesive structures of Salmonella enterica and pathogenic Escherichic		
	coli on the adhesion to salad		
13:00 – 13:15	Kristina Eißenberger		
	Internalization of enterohemorrhagic Escherichia coli into the roots of lettuce plants		
13:30 – 14:30	Lunch		
14:30 – 17:00	Stakeholder meeting		
14.50 17.00	AG Human Pathogens on Crop Plants meeting with COST members and EFSA		
	(for <b>all participants</b> of the workshop)		
14.30 - 14.50	Mieke Uyttendaele, COST HUPlantControl		
	Facilitating communication on when plant becomes food and microbes become pathogens		
14.50 - 15.10	Sven Jechalke, JLU Giessen		
17.30 - 13.10	Uptake of <i>Salmonella</i> and <i>E. coil</i> into crop plants; the <i>plantinfect</i> consortium		
15.10 - 15.30	Charles Franz, MRI Kiel		
13.10 - 13.30	Human pathogens and antibiotic-resistant enterobacteria in fresh produce		
15.30 - 15.35	Mieke Uyttendaele		
13.30 - 13.33	Introduction of topics for discussion		
15.35 - 17.00	Open discussion		
	(in English/German)		
	, , ,		
17:30 – 18:30	Guided visit to Botanical Garden Berlin		
19:00	Free evening		





# Day 3

#### Wednesday 14.03.2018

9:00	Registration
9:00 – 12:00	MC Core Meeting (for CA16110 <b>MC members only</b> )
12:00 – 13:30	Lunch
14:00	Departure

### **Abstracts**

### **TAKLS**





#### 11.

Effect of irrigation with urban wastewater and sustainable soil management on the presence and persistence of potential HPMOs in olive trees

**Adriano Sofo**<sup>a</sup>, Alba Mininni<sup>b</sup>, Catia Fausto<sup>b</sup>, Bartolomeo Dichio<sup>b</sup>, Cristos Xiloyannis<sup>b</sup>, Silvia Pascazio<sup>c</sup>, Marina Scagliola<sup>c</sup>, Carmine Crecchio<sup>c</sup>

- <sup>a</sup> School of Agricultural, Forestry, Food and Environmental Sciences (SAFE), Università degli Studi della Basilicata, Viale dell'Ateneo Lucano, 10 85100 Potenza, Italy.
- <sup>b</sup> Department of European and Mediterranean Cultures: Architecture, Environment and Cultural Heritage (DiCEM), Università degli Studi della Basilicata, Via San Rocco, 3 75100, Matera, Italy.
- <sup>c</sup> Department of Soil, Plant and Food Sciences (DiSSPA), Università degli Studi di Bari "Aldo Moro", Via Amendola, 165 70126 Bari, Italy.

Under suitable conditions, low-quality, urban wastewater is an additional water resource for irrigation in water-scarce environments but its use in agriculture requires a careful monitoring of a range of hygiene parameters, including HPMOs. Culture-based and DNA-based microbiological analyses on soil, xylem sap, leaves and fruits were carried out in an olive (Olea europaea L.) grove located in Southern Italy (Basilicata region). The experimental grove has been managed in two plots for 18 years. The first plot (IRR), non-tilled, was drip irrigated daily with reclaimed wastewater (293 mm yr<sup>-1</sup>). The second plot (N-IRR) was unirrigated (i.e. rainfed) and subject to conventional soil and plant management. Escherichia coli concentration in the wastewater varied considerably, being frequently above the stringent Italian mandatory limit of 10 CFU 100 mL<sup>-1</sup> and also the WHO limit of 1000 MPN 100 mL<sup>-1</sup>. A detailed metagenomic analysis revealed slight increases in other potential HPMOs belonging to the Enterobacteriaceae, Pseumonadaceae and Clostridiaceae families, occasionally observed in IRR soil and plant compartments. In the IRR plot, no significant HPMO bacterial contamination was recorded in the surface and pulp of the fruits harvested directly from the canopy or sampled from the ground. The results confirmed that fertigation urban wastewater did not cause significant increases or persistence of bacterial HPMOs in the soil and plants of the IRR plot and that, among the ecological niches where HPMOs live, xylem sap could be a reservoir of bacteria, mainly deriving from the soil but partially also from the canopy.

