3rd International Workshop on **Interactions between crop plants and human pathogens**

HU Berlin, Dahlem 12 -14.03.2018

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COST is supported by the EU Framework Programme Horizon 2020

3rd 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 agro-production, such as soils, water and fertilizers can play a role in contamination of plants, but micro-organisms 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)

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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

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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 – 13:30	Lunch	13:00 – 14:30	Lunch	12:00 – 13:30	Lunch pause
13:30 – 14:30	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
14:30 – 15:15	CEBAS Presentation **Ana Allende Prieto** Risk posed by pathogens in food of non-animal origin: what is going on lately at the CEBAS-CSIC?				
15:55 – 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		

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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

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Stakeholder Meeting

Tuesday 13.03.2018

Detailed program of stakeholder meeting

14:30 – 17:00	Stakeholder meeting 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 *plantinfect* 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.

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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

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Session schedule

Day 1

Monday 12.03.2018

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** Microbiota at different stages of industrial processing of ready to eat salad
11:45 – 12:00	**Lofti Fki** Human pathogenic microorganisms in in vitro plant tissue cultures: the state of the art
12:00 – 12:15	**Silke Ruppel** The phyllosphere of *Lepidium sativum* – 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 – 13:30	Lunch
13:30 – 14:15	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 – 15:15	CEBAS Presentation **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. **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** 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 plant surfaces
17:00 – 17:15	**Julia Aguilera** Dual expression of the *Salmonella* effector SrfJ in mammalian cells and plants
17:15 – 17:30	**Ilana Fratty** Cellulases of *Salmonella* Typhimurium and their role in Salmonella-plants interactions
19:00	Conference Dinner - Restaurant Englers

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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** Tracing the transferable resistome in bacteria of the agro-ecosystem
9:45 – 10:15	**Tomislav Cernava** Assessment of antibiotic resistances in the arugula microbiome
10:15 – 10:45	**Robert Czajkowski** Lytic bacteriophages in agricultural environment - a (new or rediscovered?) way to control bacterial infections in crop plants
10:45 – 11:00	**Alex Samusev** Alternative view on the LPS importance in the *Salmonella*-plants interactions
11:00 – 11:15	**Gregor Fiedler** Characteristics of ESBL-producing *Enterobacteria* isolated from sprouts
	coffee
11:30 – 13:00	**Session IV** Internalization of human pathogens in plant tissues. **Chair: Adam Schikora**
11:30 – 12:00	**Adam Schikora** 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 *Escherichia 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** AG Human Pathogens on Crop Plants meeting with COST members and EFSA (for **all participants** 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 Uptake of *Salmonella* and *E. coil* into crop plants; the *plantinfect* consortium
15.10 - 15.30	**Charles Franz**, MRI Kiel Human pathogens and antibiotic-resistant enterobacteria in fresh produce
15:30 – 16:00	**coffee**
16.00 - 16.15	**Mieke Uyttendaele** Introduction of topics for discussion
16.15 - 17.00	Open discussion (in English/German)
17:30 – 18:30	Guided visit to Botanical Garden Berlin
19:00	Free evening

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Day 3

Wednesday 14.03.2018

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

Abstracts

TAKLS

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1.
Produce safety from farm to table: Rare opportunities for opportunists at various scales

Maria Brandl

Produce Safety and Microbiology Research Unit
USDA, ARS, WRRC, 800 Buchanan Street Albany, CA 94710, USA

Produce contamination with enteric pathogens continues to cause outbreaks despite extensive efforts in preventative crop management and post-harvest handling of product. Several field studies have demonstrated that human enteric pathogens survive, albeit poorly and generally for only short periods of time, after their inoculation onto plants. These field studies, as well as quality testing by the industry in the field and during processing, also reveal that enterics persist on plants in a random, rare, and sporadic manner. Our laboratory investigations show that opportunities exist for human pathogen survival and proliferation in the phyllopshere of common culprits such as lettuce and cilantro. At the scale of single bacterial cells, we have observed a highly heterogeneous distribution of enterics in the phyllosphere and the consistent formation of large cell aggregates at sites of injury on damaged, infected, or cut leaves. Our transcriptomic and related experimental data uncovered a high adaptation of *Salmonella enterica* and *E. coli* O157:H7 to the nutritional environment and physicochemical stresses that may occur at microsites on intact leaves and in wounded leaf tissue. This physiological plasticity, which is also driven by genetic polymorphism at the intra- and inter-specific levels of enteric cell populations, must be considered in our pursuit of an enhanced understanding of the complex ecology of human pathogens on plants in order to better predict and control the probability of food-borne illness associated with crop contamination events.

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2.
Plants as a reservoir for emerging multi-resistant human pathogens

Gabriele Berg

Institute of Environmental Biotechnology, Graz University of Technology, Petersgasse 12, 8010 Graz, Austria

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During the last years, the number of human infections caused by bacterial pathogens of plant's origin has increased dramatically. Generally, they can be divided into two groups: i) opportunistic pathogens that cause the majority of health-care associated infections (HAIs) world-wide and ii) human pathogens that cause severe food-borne diseases. This presentation will focus on the first group, which includes opportunistic pathogens such as *Acinetobacter, Burkholderia, Enterobacter, Herbaspirillum, Ochrobactrum, Pseudomonas, Ralstonia, Staphylococcus, Stenotrophomonas* from the rhizosphere. Mechanisms responsible for colonization of the rhizosphere and their antagonistic activity against plant pathogens are similar to those responsible for colonization of human tissues and pathogenicity. Multiple resistances against antibiotics are not only found with clinical strains but also with strains isolated from the plant. High competition, the occurrence of diverse antibiotics and antimicrobial plant secondary metabolites on and in the plant, and enhanced horizontal gene transfer rates in these microenvironments appear to contribute to the high levels of natural resistances. Therefore, the top 3 of the WHO priority list to fight against antibiotic resistances are of plant origin. A combination of multi-omics with innovative single cell techniques allowed a deeper insight into the plant microbiome and their members with human pathogenicity.

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3.
The risk to consumers by internalised human pathogens in vegetables

Bernhard Merget[1], Norval Strachan[2], Ken Forbes[2], Fiona Brennan[3], **Nicola Holden**[1]

(1) The James Hutton Institute, Dundee, DD2 5DA UK
(2) The University of Aberdeen, Aberdeen, AB24 3FX, UK
(3) Teagasc, Johnstown Castle, Co. Wexford. Y35 Y521

Food-borne pathogens, such as *Salmonella* or Shigatoxigenic *Escherichia coli* (STEC) are able to internalise into plants. Once established *in planta* they can persist for several weeks and are even reported to grow internally. These internal bacteria are not removed by current mitigation steps in the industry, such as chlorite in washing water, and so pose a food-borne hazard on fresh produce. Yet current risk assessments and food safety regulation do not take internalised bacteria into account.
The aim of this work was to create a risk framework for STEC in green leafy vegetables, to complement knowledge gaps with novel data and to develop a risk assessment considering the risk from-farm-to-fork in the United Kingdom (UK). Bacterial growth characterisation, underpinned by metabolite studies of different tissues of lettuce and spinach were implemented in a novel quantitative risk assessment (QRA) to estimate the risk by consuming ready-to-eat (RTE) salad bags from the supermarkets.
Bacterial growth was significantly affected by different plant host species and plant tissue types. The tissues contained a diverse range of metabolites, where the only common driver of bacterial growth was the broad group of saccharides. Growth rates mirrored *in planta* colonisation. The data was implemented into the farm-to-fork QRA, showing an estimated probability of illness to be $1.51 * 10^{-5}$ and $2.66 * 10^{-5}$ for spinach and lettuce, respectively. This was main driver was the presence of internalised bacteria, responsible for 81 % and 91 % of reported illnesses, respectively.
Internalised STEC is not influenced by current mitigation steps and appears to be responsible for the vast majority of reported illnesses associated with the consumption of RTE salads from supermarkets in the UK.

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4.
Microbiota at different stages of industrial processing of ready to eat salad

Charles Franz

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5.
Human pathogenic microorganisms in *in vitro* plant tissue cultures: the state of the art

A. Nasri[1], H.M. Shumacher[2], E. Baklouti[1], A. Ben Romdhane[1], M. Maalej[1], N. Drira[1], S. Tounsi[3], and **L. Fki[1]**

[1] Laboratory of Plant Biotechnology, Faculty of Sciences of Sfax- Route Sokra BP 1171, 3000 Sfax, Tunisia, University of Sfax,Tunisia
[2] Leibniz Institute DSMZ German Collection of Microorganisms and Cell Cultures GmbH, Inhoffenstr. 7b, 38124 Braunschweig, Germany
[3] Laboratory of Biopesticides, Centre of Biotechnology of Sfax-Route Sidi Mansour, Sfax Tunisia

Plant tissue culture consists in cultivating plant cells on a synthetic nutrient media under sterile and controlled conditions. Plant tissue culturists have tried, often without success, to eliminate all microorganisms from *in vitro* cultures. Indeed, establishment of axenic *in vitro* plant cultures doesn't seem easy to accomplish. Recent studies have shown that there is a risk of the subsistence of human pathogens such as Mycobacteria in plant tissue cultures. Bacterial strains pathogenic to humans can be stably maintained in the *in vitro* tissue culture. The broadening of bacterial environments creates ecological and genetic risks leading to the necessity of careful monitoring of endophytic communities in plant tissue culture. Therefore, tissue culturists must consider a possible subsistence of human pathogenic microorganisms in all established *in vitro* tissue cultures.

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6.
The phyllosphere of *Lepidium sativum* – a habitat of human probiotic or pathogenic bacteria?

S. Ruppel[1], S. Patz[3], M. Becker[1], B. Berger[4]; A.-C. Scherwinski[1], N. Hegazi[2]

[1]Leibniz-Institute of Vegetable- and Ornamental Crops Großbeeren / Erfurt e.V., 14979 Großbeeren, Germany
[2]Cairo University, Faculty of Agriculture, Environmental Studies and Research Unit, Department of Microbiology, Giza, Egypt

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7.

To be or not to be a pathogen: Differentiate true virulence factors of bacteria from false positives

Becker, Matthias[1]; Becker, Yvonne[1,3]; Ruppel, Silke[1] und Patz, Sascha[1,2]

[1]Leibniz Institute of Vegetable and Ornamental Crops, Grossbeeren, Germany ; [2]Algorithms in Bioinformatics, Center for Bioinformatics, University of Tübingen, Tübingen, Germany ; [3]Institute for Epidemiology and Pathogen Diagnostics, Julius Kühn-Institute – Federal Research Centre for Cultivated Plants, Braunschweig, Germany

The application of beneficial bacteria in agriculture is a much appreciated alternative to fertilizers and pesticides. However, any promising bio-inoculant may turn into a threat to human health if the bacterium is able to switch from plant host to animal host. The recent worldwide discovery of plant growth-promoting *Kosakonia radicincitans* in a large variety of crop plants suggests that this species confers significant influence on plants, both in terms of yield increase and product quality improvement. However, in 2017 the first case of *K. radicincitans* causing a human bloodstream infection was reported. Now we have compared the genomic composition of this human pathogen to plant growth-promoting strains of *K. radicincitans* and found significant differences. We learned that particular genomic features are still considered virulence factors of pathogenic bacteria, though they have meanwhile been associated with important characteristics of non-pathogenic bacteria. Here I employ the case study of *K. radicincitans* to exemplify the need for more sophisticated research on beneficial microbe-plant interaction and for close collaboration of scientists working on human pathogens and scientists developing bio-inoculants for agriculture.

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8.
Risk posed by pathogens in food of non-animal origin (FoNAO): EU outbreak data analysis and risk ranking (2011-2015)

Maria Teresa da Silva Felicio

BIOCONTAM Unit, European Food Safety Authority, Parma, Italy (EFSA)

After the German STEC O104:H4 outbreak in 2011, EFSA received a mandate from the European Commission to: (i) assess the public health risk posed by pathogens that may contaminate food of non-animal origin (FoNAO), (ii) compare the incidence of foodborne human cases linked to FoNAO and food of animal origin (FoAO) and (iii) identify and rank specific food/pathogen combinations most often linked to foodborne human cases from FoNAO in the EU. A semi-quantitative model was developed using seven criteria: 1) strength of associations between food and pathogen based on the foodborne outbreak data from EU Zoonoses Monitoring (2007-11), 2) incidence of illness, 3) burden of disease, 4) dose-response relationship, 5) consumption, 6) prevalence of contamination and 7) pathogen growth potential during shelf life. Despite the inherent assumptions and limitations, this risk ranking model is considered valuable, as it allowed identifying food/pathogen combinations most often linked to foodborne human cases originating from FoNAO in the EU. Five groups of top ranking food/pathogen combinations were recognised, namely: *Salmonella* and Norovirus in leafy greens eaten raw; *Salmonella* and Norovirus in berries; *Salmonella* and Norovirus in tomatoes; *Salmonella* in melons; and *Salmonella, Yersinia, Shigella* and Norovirus in bulb and stem vegetables, and carrots. The main risk factors as well as possible specific mitigation options for these food/pathogen combinations were discussed. To assist future microbiological risk assessments in this area, considerations should be given to the collection of additional information on how food has been processed, stored and prepared as part of related data collection exercises.

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9.
Risk posed by pathogens in food of non-animal origin: what is going on lately at the CEBAS-CSIC?

Ana Allende

CEBAS-CSIC Centro de Edafología y Biología Aplicada del Segura

The EFSA published seven scientific opinions of the BIOHAZ Panel focused on the risk posed by pathogens in different food of non-animal origin between 2011 and 2014. This was the first time EFSA was focused on the identification of the main risk factors for specific fresh produce/pathogen combinations. At that moment, there was scarce information available about how different agricultural practices influenced the prevalence and concentrations of pathogens in fresh produce in the EU. Most of the available data originated from countries outside Europe, such as USA. Relevant information coming from an European Project "VEG-i-TRADE" was made available right after the publications of these opinions and since then, also several research projects have been funded to determine the impact of different agricultural practices or the use of specific intervention strategies on the contamination of fresh produce with biological hazards. This talk aims to summarise relevant information obtained in the last 4 years on the risk posed by pathogens in food of non-animal origin, from different research projects run at the CEBAS-CSIC. Several studies will be presented including: 1) risk factors associated to the use of different irrigation water sources; 2) development of a quantitative microbial contamination model to investigate how the selection of the irrigation water sources affect the *E. coli* loads in leafy greens at harvest; 3) impact of environmental conditions on the growth of *Salmonella*; 4) optimization of the operational conditions of fresh produce processing lines at commercial scale; 5) potential overestimation of the anti-microbial capacity of commercial sanitizers during water disinfection or washing.

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10.
How do environmental factors affect infection of plants by the Human Pathogenic Microorganisms (HPMO)?

Katarzyna Hrynkiewicz

[1] Department of Microbiology, Faculty of Biology and Environmental Protection, Nicolaus Copernicus University, Poland
[2] Centre of Modern Interdisciplinary Technologies, NCU, Poland

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Human pathogenic microorganisms (HPMO) may be present in many different environments (e.g. water, soil) and contaminate edible plants, posing a significant risks for human health. Monitoring of the quality of water used for irrigation of plants, soils and finally fresh edible plants is part of a broad control of the microbiological quality. However, the use of the fecal bacteria indicators (e.g. *Salmonella* and *Shigella*) have only a limited predictive efficiency and does not allow to detect all potentially pathogenic microorganisms.
The biotic and abiotic factors play a key role and can affect the survival and the infectivity of HPMO in the environment. Differentiated physico-chemical soil properties, e.g. pollution caused by anthropogenic activity, may influence the diversity and survival HPMO in the environment. Moreover, different plant species or cultivars can show various tolerance to potential HPMO infection, resulting from morphological and anatomical differences, biochemical defense mechanisms or plant fitness. On the other hand, rhizosphere soil or plant microbiome, as a reservoir of numerous and diverse microorganisms, may contain microorganisms participating in the biological control of HPMO. Therefore, several abiotic and biotic factors and their combined effects should be considered and confirmed to evaluate persistence and the potential infection of plants with HPMO in natural conditions, to assess safety of fresh produce.
Some of the outcomes from the environmental studies will be presented during this talk, as well as preliminary studies on selected HPMO and plant-associated microorganisms, as potential microorganisms participating in the biological control of HPMO.

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11.

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

Adriano Sofo[a], Alba Mininni[b], Catia Fausto[b], Bartolomeo Dichio[b], Cristos Xiloyannis[b], Silvia Pascazio[c], Marina Scagliola[c], Carmine Crecchio[c]

[a] School of Agricultural, Forestry, Food and Environmental Sciences (SAFE), Università degli Studi della Basilicata, Viale dell'Ateneo Lucano, 10 – 85100 Potenza, Italy.
[b] Department of European and Mediterranean Cultures: Architecture, Environment and Cultural Heritage (DiCEM), Università degli Studi della Basilicata, Via San Rocco, 3 – 75100, Matera, Italy.
[c] 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^{-1}). 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^{-1} and also the WHO limit of 1000 MPN 100 mL^{-1}. 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.

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12.

To be or not to be inside: A complex role of bacterial factors for endo- or epiphytic colonization

Andreas Beust, Xun Jiang, Marta Marszalkowska, Theresa Dinse, Martin Schäfer, Abhijit Sarkar and **Barbara Reinhold-Hurek**

Department of Microbe-Plant Interactions, University of Bremen, PO. Box 334040, 28334 Bremen, Germany

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The diazotrophic model endophyte of grasses, Azoarcus sp. strain BH72, colonizes roots of its original host plant Kallar grass and rice in similar patterns. The molecular mechanisms by which endophytes interact with their host are not yet well understood. How do bacteria adapt to their endophytic lifestyle in comparison to free-living growth, and which proteins do they require for endophytic competence?

Among the bacterial factors required for establishment in the endophytic compartment, attachments factors and protein secretion systems of Type 6 (T6SS) will be covered here. In a transcriptome microarray study, gene expression of azo1653 and azo1684 was found to be responsive O_2 concentration. Mutational analysis revealed that both encoded proteins, putative attachment factors, have a complex role in different steps of colonization. They have a negative impact on rhizosphere competence (surface colonization), however contribute to endophytic establishment inside roots. Another important bacterial tool appears to be the type VI secretion system, which is known to be involved in other bacteria in the interaction between different bacteria or between bacteria and their eukaryotic hosts. The genome of Azoarcus contains two gene clusters encoding for putative T6SSs, termed sci and imp. Secretion of the T6SS hallmark protein HCP was shown previously. Here we unraveled the structural components and expression patterns of the T6SSs in more detail. The Sci-system appeared to be constitutively active. For the Sci-system, TagF, a homolog to a Pseudomonas aeruginosa inhibitor of T6SS protein secretion, led to hypersecretion of Hcp also in Azoarcus strain BH72. However, even then we found no evidence of the Imp-system secreting its cognate Hcp protein, although the respective genes were strongly induced under conditions of nitrogen fixation. Surprisingly, strain BH72 carrying a gene knockout in this apparently inactive Imp-system showed a strong reduction in endophytic rice root colonization, indicating it is important for the interaction with the host. To visualize the secretion process, we constructed VipA-sfGFP fusions for both T6SSs, and can thus follow the process of active sectretion in vivo. Regulatory cues and the role of T6SS in endophyte-plant interaction will be discussed.

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13.

The impact of *Salmonella* type III secretion effectors on plant defense-related genes and the persistence within plant host

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Salmonella is able to cause disease in humans and animals and also to colonize plants. It usually enters agricultural environments *via* animal faeces or surface water used for irrigation. Recent reports show that *Salmonella enterica* is able to colonize a variety of plant species and organs; hence it can cause disease outbreaks and result in severe economic losses. For successful plant colonization, *Salmonella* needs to attach and adhere to plant surfaces. This might cause host cytoskeleton changes leading to bacterial entry. Importantly, *Salmonella* originating from plants maintains its virulence in animals. Thus, plants might be an alternative host for *Salmonella* and can play a role in its transmission towards animals. Nevertheless, the targets of *Salmonella* effectors in plant cells are not well characterized. Therefore, the aim of our work was to investigate the impact of chosen effectors on the plant immune system, as well as their role in *Salmonella* survival inside the different plant hosts.
We found that *Salmonella* can survive up to 14 days post inoculation inside the plant without a significant change in the plant phenotype. In addition, our results show a higher and longer last expression of some plant defense-related genes after inoculation with particular effector mutants. Our results give also rise to a hypothesis suggesting the existence of redundant effectors. *Salmonella* might use them to compensate the deficiency of particular (other) effectors. However, this hypothesis requires further experimental tests.

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14.

Dual expression of the *Salmonella* effector SrfJ in mammalian cells and plants

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Salmonella is a Gram-negative facultative intracellular pathogen, which belongs to the family Enterobacteriaceae. Its pathogenic properties are partially a consequence of virulence genes that encode effector proteins. SrfJ is an effector of the *Salmonella* pathogenicity island 2-encoded type III secretion system. *Salmonella enterica* serovar Typhimurium expresses *srfJ* under two disparate conditions: media with low Mg and low pH, imitating intravacuolar conditions, and media with *myo*-inositol, a carbohydrate that can be used by *Salmonella* as sole carbon source. We investigated the molecular basis for this dual regulation. The expression of *srfJ* is under the control of two promoters that are induced by different conditions. A proximal promoter, P*srfJ*, responds to intravacuolar signals and it is positively regulated by SsrB and PhoP and negatively regulated by RcsB. A second distant promoter, P*iolE*, is negatively regulated by the *myo*-inositol island repressor IolR. Interestingly, our results indicate that the proximal promoter is specifically active inside mammalian cells whereas the distant one is expressed upon *Salmonella* colonization of plants. Importantly, we also found that inappropriate expression of *srfJ* leads to reduced proliferation inside macrophages whereas lack of *srfJ* expression increases survival and decreases activation of defense responses in plants. These observations suggest that SrfJ may relevant in the interplay between *Salmonella* and hosts of different kingdoms.

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15.

Cellulases of *Salmonella* Typhimurium and their role in *Salmonella*-plants interactions

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Consumption of fresh fruit, vegetables and leafy greens may be associated with pathogen-related outbreaks, especially *Salmonella enterica* serovars. Mechanisms of attachment and survival of *Salmonella* on plants are under extensive investigation, but the mechanism of *Salmonellas*' invasion to plants is not fully understood. Many phytopathogens facilitate invasion to the host by using Cell Wall Degrading Enzymes (CWDE). This study examines the possible functions of CWDE in *Salmonella* Typhimurium and its importance for bacterial survival on plants.

Bioinformatics search of the *S*. Typhimurium genome resulted in two genes, *bcsZ* and *bcsG* encoding for proteins with a putative cellulase activity. After purifying recombinant BcsZ and BcsG, we observed hydrolytic activity of Carboxymethylcellulose (CMC) on CMC-agar plates and high cellulase activity in saline using the Bicinchoninic acid assay. Our next challenge was to find whether the cellulases BcsZ and BcsG affect the survival of *Salmonella* on and in plants. Irrigation or injection of parsley leaves with bacterial solutions containing *S*. Typhimurium-overexpressing BcsZ strain (pBcsZ) in compare to the *bcsZ* knockout mutat (ΔBcsZ), resulted in significant higher bacterial survival of pBcsZ after 7 and 14 days, which means BcsZ affects the epiphytic and endophytic survival of *Salmonella* in plants. Furthermore, injection of pBcsZ or the purified BcsZ to parsley leaves resulted in necrotic areas near the injection spots, meaning the plant immune system was activated and reacted with hypersensitive response (HR) symptoms.

As for irrigation of parsley plants with *Salmonella*-overexpressing BcsG (pBcsG), we observed higher bacterial survival on irrigated parsley leaves with pBcsG comparing to parsley leaves irrigated with the wild type *Salmonella* (WT). However, parsley leaves irrigated with the knockout mutant of *bcsG* (ΔBcsG) had similar *Salmonella* levels comparing to pBcsG, and similar results were shown in endophytic survival experiments between pBcsG and ΔBcsG.

Injection of pBcsG to parsley leaves did not result in HR symptoms but injection of the recombinant BcsG resulted in HR symptoms. Overall, the results lead us to suggest that the plant immune system can be triggered by degradation of plant cell wall caused by BcsZ and BcsG function. Interestingly, activity experiments of the *bcsG* promoter demonstrated high induction in the presence of plant components such as cellulose, xylan and parsley extraction, which means BcsG is highly expressed in the first days after sensing the presence of plant component.

This study reveals, for the first time, that BcsZ and BcsG in *S.* Typhimurium are cellulases playing an important role in plant-bacteria interactions, affecting epiphytic and endophytic survival of *S.* Typhimurium on plans and triggering the plant's innate immune response.

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16.
Tracing the transferable resistome in bacteria of the agro-ecosystem

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Sequencing of bacterial genomes showed that a substantial proportion of the sequences seemed to be horizontally acquired. Nowadays it is commonly accepted that genes conferring traits that allow a rapid adaptation to changing environmental conditions and to colonize new ecological niches are often localized on mobile genetic elements such as plasmids. It is assumed that plasmids played a major role in disseminating genes conferring resistances towards antibiotics, metal compounds or disinfectants (called transferable resistome) but they can carry partial or complete degradative pathways. Plasmids are typically carried in a small proportion of the cells of a given population which ensure the rapid adaptation to stress but also to the respective niche. This can be seen as average fitness increase due to genetic variance and variance of fitness. Our understanding of the ecology of plasmid-mediated gene transfer and what is driving the capture of accessory gene loads is still incomplete and often plasmids can only be detected when their hosts become numerically abundant, e.g. under selective pressure.
In the last decades several cultivation independent methods were employed to study plasmids in in the agro-ecosystem. Using the example of the broad-host range plasmids belonging to the IncP-1 group recent insights into the environmental dissemination, evolution and potential role of IncP-1 plasmids in spreading resistance genes will be presented. Sequencing of recently captured IncP-1 plasmids from the rhizosphere revealed an unexpected diversity and suggested that plasmids are important means for driving the diversification and increase host fitness due to sequence variation.
Cultivation-dependent and independent methods were also employed to provide insights into the transferable resistome of bacteria associated with fresh produce. Our data highlight the importance of the rare microbiome of fresh produce as source of transferable antibiotic resistance genes that can be potentially transferred to human pathogens with implications for human health.

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17.
Assessment of antibiotic resistances in the arugula microbiome

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Eruca vesicaria var. *sativa* (Mill.) Thell., commonly known as arugula, is a popular raw-eaten ingredient in salads due to its peppery, pungent taste. Similar to other leafy greens, *E. sativa* is colonized by a vast diversity of microbes, which complement the holobiont's functioning. The indigenous microbiota only rarely allows foodborne pathogens to settle, indicating high competitiveness. Nevertheless, the prevalence of resistances in such microbial communities is only poorly understood. We analyzed the structure, abundance and functioning of the plant-associated microbiota in the arugula phyllosphere, rhizosphere and the corresponding bulk soil in an integrative approach. When compared to the rhizosphere, higher proportions of *Gammaproteobacteria*, including *Enterobacteriaceae* were observed in aerial plant parts. Their occurrence was verified by fluorescence *in situ* hybridization coupled with confocal laser scanning microcopy in different plant compartments. Complementary metagenomic profiling of the bacterial population indicated a higher prevalence of antibiotic resistances in plant-derived samples. We found general resistance mechanisms including various efflux pumps in the datasets, but also specific resistance mechanisms against fluoroquinolone, chloramphenicol and other antibiotics. Due to the high occurrence of *Enterobacteriaceae* in arugula samples, we screened a representative culture collection for resistances against eight common antibiotics. It was shown that more than 90% of the isolates were resistant against Ampicillin, Erythromycin, and Penicillin. Our findings suggest that antibiotic resistance is common in distinct raw-eaten plants; however the implications for human health remain unclear.

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18.

Lytic bacteriophages in agricultural environment - a (new or rediscovered?) way to control bacterial infections in crop plants

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Bacteriophages (or phages) are bacterial viruses able to infect and kill bacteria. Although phage-bacterial interactions have been studied since the beginning of their discovery, still little is known about their global ecological effect. It is generally accepted that bacteriophages are able to infect most of known bacterial species, even as far evolutionally as cyanobacteria, archaea or mycoplasmas. Phages are able to shape the structure of host population and consequently may influence virulence as well as bacterial infection process in animals and plants.

We investigated the specific ecological interaction between plant pathogenic *Dickeya solani* and a broad host lytic bacteriophage ϕD5, both isolated in our former studies. Pectinolytic bacteria (now called soft rot *Enterobacteriaceae*: *Pectobacterium* spp. and *Dickeya* spp., formerly known as pectinolytic *Erwinias*) are ubiquitous necrotrophic bacterial pathogens that infect a large number of different plant species worldwide, including economically important crops. Despite the fact that these bacteria have been studied for more than 50 years, little is known of their corresponding predators: lytic and lysogenic bacteriophages. Up till now about 2000 bacteriophage isolates (ca. 30% of all known bacteriophages) target members of the *Enterobacteriaceae* family, however the number of characterized bacteriophages infecting *Pectobacterium* spp. and *Dickeya* spp. is much fewer and less than twenty. Likewise, the first (complete and/or draft) genomes of *Pectobacterium* spp. and *Dickeya* spp. bacteriophages were published as recently as in 2012.

This presentation acknowledges past and present work on lytic (and lysogenic) bacteriophages able to infect *Pectobacterium* spp. and *Dickeya* spp. bacteria with the major focus on isolation of (novel) lytic bacteriophages from environmental samples and their morphological, proteomic, phenotypic and phylogenetic characterization together with the use of next generation sequencing and bioinformatic technologies in analyses of bacteriophage genomes and comparative phage genomics.

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19.
Alternative view on the LPS importance in the *Salmonella*-plants interactions

Alex Samusev

In 2007, *Salmonella enterica* serovar Senftenberg was isolated as the cause of a basil outbreak in Europe. Because basil produces a variety of antimicrobial agents in the form of essential oils, it was hypothesized that *S*. Senftenberg exhibits resistance to basil oil. Indeed a clinical isolate of this outbreak was resistant to basil oil and to linalool, its major antimicrobial component. This study aims to elucidate the mechanisms of *S*. Senftenberg survival on basil plants by identifying the mechanisms of resistance to basil oil and linalool, while screening for sensitive mutants created by a transposon insertion. Our transposon library provided several linalool sensitive mutants, some of which contained a damaged *rfaG* gene. RfaG takes part in the synthesis of core oligosaccharides of the lipopolysaccharide (LPS), and damaged RfaG mutants are known to have a shorter LPS chain.
We found that RfaG mutants exhibit hyper sensitivity to linalool, basil oil, and antibiotics. Furthermore, the transposon insertion sites in the *rfaG* gene influenced bacterial phenotypes, including bacterial stress response. We observed various attachment abilities to whole basil plants and basil cell cultures among different RfaG mutants. For example, the mutant containing RfaG with 204aa length exhibited a 2.4 log CFU/g decrease in adhesion to basil cells in comparison to the wild type strain, while the mutant containing RfaG with a 26aa length decreased 1.5 log CFU/g in comparison to the wild type strain. Similar bacterial survival patterns were observed on whole basil plants. Likewise, additional differences in physiological properties and resistance between RfaG mutants were discovered.
While it is commonly thought that shortened LPS play a significant role in the increased sensitivity of the RfaG mutants, this study reveals that damage to the bacterial LPS synthesis pathway itself has a deeper, more complicated influence on bacterial survival mechanisms and on the survival of bacteria on basil plants. Better understanding of LPS-related molecular mechanisms that improve the survival of *Salmonella* on plant tissues will assist us to create a better solution for fresh products contamination.

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20.
Characteristics of ESBL-producing *Enterobacteria* isolated from sprouts

Gregor Fiedler

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21.
Colonization patterns on crop plants: same-same but different?

Sven Jechalke[1,2], Jasper Schierstaedt[1,3], Marlies Becker[1], Helena J. Barkowski[1], Rita Grosch[3], Kornelia Smalla[1] and **Adam Schikora**[1]

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The interaction between human pathogens and the plant host seems to be an active process. The recent development of knowledge in this field supports the notion that these pathogens not only survive on or within plant tissues, but also use them in a very active way as suitable environment. It is therefore very tempting to consider plants as possible alternative host for, at least some, human pathogenic bacteria and attribute to crop plants a vector function in the spread of food-borne diseases. While the mechanisms of colonization in the animal or human habitats are mostly very well understood, we are still struggling to fully understand the mechanisms used to colonize plants. Very striking is the fact that the patterns of colonization displayed by plant pathogens are somehow mimicked by human pathogens during plant colonization. Whether those patters are linked solely to the availability of nutrients is a very interesting hypothesis, which gained a lot of attention in recent years. The talk should present a small overview on our understanding of the colonization mechanisms and the niches utilized by human pathogens on and in the plant host.

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22.

Factors influencing the survival of *Salmonella enterica* in soil and the colonization of crop plants

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Salmonellosis outbreaks are increasingly associated with the consumption of contaminated raw fruits and vegetables. Contamination of produce can occur along the whole production chain also, during the plants growth. However, the knowledge about factors influencing the persistence of *Salmonella* in the plant environment and the associated colonization of plants is scarce. We analysed, the influence of preadaptation, soil type, organic fertilizer amendment and soil sterilisation on the survival of *S. enterica* serovar Typhimurium stains 14028s, and LT2, and *S.enterica* serovar Senftenberg in soil. At the same time, we analysed the colonization of lettuce, corn salad and tomato by *Salmonella* and the related plant immune responses. Preadaptation of *Salmonella* was simulated by cultivation in a new-developed lettuce medium. In summary, despite an initial decline, our data indicated that survival of *Salmonella* was higher in loamy than in sandy soil. Preadaptation promoted the survival of *Salmonella*, while competition by the indigenous soil microbial community reduced its survival. Organic fertilizer amendment had a positive effect on the survival of *Salmonella* in soil. We observed a colonization of plants at a low percentage range that seemed to be affected by the soil type, the plant as well as the *Salmonella* strain. Interestingly, the plants reacted to colonisation with an induction of defence responses that was dependent on the bacterial serovar. Together, our results indicate that *Salmonella* can persist in soil, posing a risk of fresh produce contamination. The fact that *Salmonella* use plants as alternative hosts strongly suggests that plants represent a much larger reservoir for animal pathogens than so far estimated.

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23.
High diversity of soil microbiome reduces survival of *Salmonella* in the phytosphere

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Foodborne diseases are increasingly associated with fresh fruits and vegetables and *Salmonella* was their second most frequent cause in 2015. The biological diversity of soil plays a major role in the establishment of *Salmonella* in plant environment. Different members of the microbiome can be direct antagonist or can induce plant immune system. Here, we analyzed the tripartite interactions between tomato, lettuce and corn salad plants grown under greenhouse condititions, the human pathogen *Salmonella enterica* and the soil microbial community. We observed that *Salmonella* persisted in the rhizosphere of lettuce and tomato. In contrast, its numbers declined in the rhizosphere of corn salad. Very important was the observation that reduction of microbial diversity in soil increased the ability of *Salmonella* to persist in this environment. These results clearly show a dependency between the potential of Salmonella to colonize the rhizosphere and bacterial richness as well as the high physiological plasticity of *Salmonella*. In the following, we focused on the impact of induced resistance. In greenhouse experiments we primed crop plants by inoculation of *Ensifer meliloti* to the soil close to the roots. This bacterium produces the signaling molecule *N*-acyl-homoserine-lactone which might induce resistance against *Salmonella enterica*. Our results show that priming has a negative effect on the persistence of *Salmonella*. Primed plants are able to express defense related genes earlier than unprimed plants and are able to keep stomata closed. These results indicate the potential of priming to enhance resistance against *S. enterica.*

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24.
Molecular analysis of the impact of adhesive structures of *Salmonella enterica* and pathogenic *Escherichia coli* on the adhesion to salad

Laura Elpers

Salmonella enterica serovar Typhimurium (STM) and pathogenic *Escherichia coli* strains are foodborne pathogens which causes severe diseases. The most prominent way of these pathogens to infect humans is by contaminated animal products. However they can also spread by fresh products such as salads. The mechanisms how bacteria attach to different salads and vegetables and invade into plant tissues are still unknown. In this context the large repertoire of adhesive structures involving fimbrial adhesins, non-fimbrial adhesins as well as outer membrane proteins (OMPs) and flagella are taken into account. As only few of these adhesive structures are expressed under laboratory conditions and in mammalian hosts, we hypothesize their relevance under different environmental conditions, thereby contributing to the colonization of various non-host environments. We established an assay to quantify the role of each of the various adhesins of STM in adhesion to fresh products. The adhesion assay with corn salad is applicable to other fresh products e.g. lettuce (*Lactuca sativa*). Adhesins of EHEC can also be expressed by *E. coli* laboratory strains to investigate their role in adhesion to fresh products. The impact of various adhesive structures of STM will be presented during the talk.

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25.
Internalization of enterohemorrhagic *Escherichia coli* into the roots of lettuce plants

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Within the last years an increasing number of food-associated outbreaks caused by enterohemorrhagic *Escherichia coli* (EHEC) were traced back to the consumption of contaminated fresh produce such as salads, spinach and sprouts. As bacterial contamination may occur directly on the field *via* manure, fecal contamination, irrigation or surface water, it is crucial to evaluate whether and to what extent EHEC are able to colonize plant roots. Therefore, the ability of *E. coli* O157:H7 strain Sakai to adhere to and internalize into the root tissues of lamb's lettuce was investigated under the environmental conditions of a greenhouse. Moreover, *E. coli* O104:H4 strain C227/11φcu was also investigated focusing on the potential influence of the lettuce cultivar on the adherence and internalization into the roots of either lettuce or lamb's lettuce. Upon soil contamination, approximately 10^6 colony forming units per gram root were found to be adherent irrespective of the bacterial strain and the lettuce cultivar. The number of root-internalized cells ranged from 10^2 to 10^3 cfu/g roots. Lettuce cultivar did not alter the bacterial adherence behavior whereas it showed an effect on the internalization. This study demonstrated the ability of EHEC to colonize plant roots in a lettuce cultivar dependent manner.

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POSTERS

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P1

Salmonella enterica persistence in soil amended with sewage sludge compost and its impact on the soil microbiome

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Many outbreaks of foodborne illnesses caused by *Salmonella enterica* highlight the ability of this human pathogen to adapt to different environments. While it is evident that contamination of plants occurs, the source of contamination remains often unclear. If present in soil, weather conditions and heavy irrigation can cause soil splashing on the upper part of the cultivated plant. Adherence and internalization in plant tissue are the consequences. Also, available nutrients and indigenous microbial populations play an important role in human pathogen persistence. Persistence of *Salmonella enterica* ser. Seftenberg and Typhimurium 14028s was determined experimentally in diluvial sandy soil with or without sewage sludge compost amendment. *Salmonella* strains were introduced in soil and counted by direct plating up to 56 days post planting. Additionally, the potential of transfer from soil to plant was determined by detecting *Salmonella enterica* on the plant leafs by enrichment. Chinese cabbage (*Brassica rapa* L. subspecies *pekinesis) was* chosen as the model organism. The effect of the addition of salmonella on the native microbiome was studied using DGGE from the extracted total community DNA. The obtained results suggest that the persistence of Salmonella in soil could be enhanced by the addition of sewage sludge. The presence of *Salmonella enterica* could be detected in the upper part of the plant, especially in the early stage of plant development.

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P2

Detection and Isolation of Shiga toxin-producing *Escherichia coli* from Sprouts with the Acid Treatment: Experience from a Proficiency Test

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The plant matrix comprises a special challenge for the detection and isolation of Shiga toxin-producing *Escherichia coli* (STEC) from fresh plant foodstuff especially from fresh sprouts. Here, high numbers of plant microbiota might mask or overgrow STEC during the initial enrichment procedure. As STEC is ascribed a low infectious dose, an effective inhibition of the plant microbiota during the enrichment step of a method for STEC detection and isolation is required. An acid treatment step during enrichment was shown (e.g. Fedio et al. 2012, Verhaegen et al. 2016) to improve the detection and isolation of STEC from sprouts. Therefore, we adjusted and integrated an acid treatment step into a method, which is validated for the detection and isolation of STEC from fresh plant matrices (except sprouts). A proficiency test for the detection and isolation of STEC from sprouts was organized with 22 laboratories taking part and 16 of them testing the acid method.
Two STEC isolates from the strain collection of the National Reference Laboratory for *Escherichia coli* were chosen: BfR-EC 14434 (serotype O133:H25, *stx2d*, originally isolated from sprouts) and BfR-EC 16015 (serotype O26:H11, *stx1a, stx2a, eae, ehly*, originally isolated from salad) were used for artificial contamination at a low (approx. 50 Cfu / 25 g sprouts) and a high (approx. 500 Cfu / 25 g sprouts) dose. As matrix, 25 g of fresh mung bean sprouts were used for each sample. One set consisted of six samples. Four samples were spiked with one of the above mentioned isolates at a high or low dose and two were left unspiked. The laboratories applied a method of their choice or/and the above mentioned acid method for the detection and isolation of STEC. The protocol for the acid method was provided.
In sum, the sensitivity of detection (methods chosen by the labs) was 70 % and 32 % of isolation. The sensitivity of detection (acid method) was 92 % and 88 % of isolation. In sum, the specificity of detection (methods chosen by the labs) was 95 % and 100 % of isolation. The specificity of detection (acid method) was 97 % and 100% of isolation.
In this proficiency test the acid method was shown to clearly increase the sensitivity and specificity of detection and especially isolation of two different STEC strains spiked at different concentrations in fresh mung bean sprouts. Different laboratories were able to apply the method successfully. This shows the efficiency, sensitivity, specificity and reproducibility of the acid method in this context. Especially the improved isolation of STEC here is important, as in general STEC detection needs to be verified by isolation in official investigations. General acid resistance tests of representative STEC strains are in progress.

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P3

Systemic colonization of clover (*Trifolium repens*) by *Clostridium botulinum* strain 2301

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In recent years, cases of botulism in cattle and other farm animals and also in farmers increased dramatically. It was proposed, that these cases could be affiliated with the spreading of compost or other organic manures contaminated with *Clostridium botulinum* spores on farm land. Thus, soils and fodder plants and finally farm animals could be contaminated. In this study we investigated the colonization behavior and interaction of the botulinum neurotoxin (BoNT D) producing *C. botulinum* strain 2301 and the non-toxin producing *Clostridium sporogenes* strain 1739 on clover (*Trifolium repens*).

The experiments included field as well as phytochamber experiments applying axenic and additionally soil based systems under controlled conditions. Plants were harvested and divided into root and shoot parts for further DNA isolation and PCR assays; subsamples were fixed for fluorescence *in situ* hybridization (FISH) analysis in combination with confocal laser scanning microscopy (CLSM).

To target *C. botulinum* and *C. sporogenes*, 16S rDNA directed primers were used and to specifically detect *C. botulinum*, BoNT D toxin genes targeted primers, using a multiplex PCR approach, were applied.

Our results demonstrate an effective colonization of roots and shoots of clover by *C. botulinum* strain 2301 and *C. sporogenes* strain 1739. Detailed analysis of colonization behavior showed that *C. botulinum* can occur as individual cells, in cell clusters and in microcolonies within the rhizosphere, lateral roots and within the root tissue of clover. In addition, we observed significant differences in the growth behavior of clover plants when inoculated with Clostridia spores, indicating a plant growth promoting effect. Inoculated plants showed an increased growth index (shoot size, wet and dry weight) and an enlarged root system, which suggests the involvement of phytohormonal effects induced by the systemic colonization of clover by *C. botulinum* strain 2301.

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P4

Relationship between Cultivable and VBNC *E. coli* Levels and Presence of Pathogens in Surface Irrigation Water

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Irrigation water has been identified as a major risk factor for fresh produce contamination with foodborne pathogens during primary production. Mostly due to the low prevalence of foodborne pathogens, fecal indicators have been typically used as a tool to predict potential contamination. Correlations between fecal indicator microorganisms and foodborne pathogens are not easy to establish and efforts have been made to select better indicators, including the use of cultivation-dependent and qPCR-based assays. In the present study, three types of irrigation water (water reservoir, drainage ditches and canals) were monthly monitored for one year. Microbial analyses included the enumeration of cultivable and viable but non-cultivable (VBNC) *E. coli* using the PMA-qPCR technique and pathogenic microorganisms including Shiga toxigenic *E. coli* (O157:H7, O26, O103, O111 and O145) and *Salmonella* spp. Surface water obtained from drainage ditches showed the highest levels of *E coli*. The levels of cultivable and VBNC *E. coli* were very similar in most of the samples, except for those subjected to chemical treatments, indicating the presence of stressed cells. Samples positive for the presence of foodborne pathogens showed higher levels of both cultivable and VBNC *E. coli*, demonstrating the suitability of *E. coli* as an indicator microorganism to determine the potential presence of fecal contamination.

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P5

Endophytes of *Salicornia europaea* L. and human pathogenic bacteria

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The increase in consumption of fresh vegetables increases the number of disease reports caused by human pathogenic microorganisms (HPMO), e.g. *E. coli* 0157:H7, *Salmonella* spp., *Shigella* spp., *Listeria monocytogenes*, *Clostridium botulinum*, *Bacillus cereus.* The presence of HPMOs in crops can be a consequence of using natural fertilizers and contaminated water for watering, but also wild animals living in natural conditions.

The aim of the study was to determine the taxonomic diversity of *S. europaea* root endophytes isolated from the two research sites with different salinity level based on cultured (16S rDNA sequences analysis) and uncultured (metagenomic analysis of the V3-V4 16S rDNA gene region) approach. *S. europaea* is edible halophytic plant growing on wasteland and the possible occurrence of HPMOs can be the result of wild animals occurrence. The results of our study based on culture-dependent techniques revealed the presence of root endophytic strains belonging to Firmicutes, Proteobacteria and Actinobacteria. Metagenomic analysis confirmed the dominance of the above mentioned phyla and additionally occurrence Planctomycetes, Actinobacteria, Chloroflexi, Acidobacteria, Verrucomicrobia, OD1, Chlamydiae, TM7 and Fibrobacteres. Moreover, our results confirmed the presence of potential HPMOs e.g.: *Williamsia* sp., *Bacillus cereus*, *Bacillus thuringensis*, *Bacillus weihenstephanensis*, *Mycobacterium* sp.

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Safety risks linked to home sprouting modules

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Despite the sprouts associated outbreaks, growing and consumption of raw sprouted seeds is associated with a healthy lifestyle in Scandinavia, and different types of sprouting modules for home sprouts production are available on the market. During sprouting, ideal conditions for seed emergence, but also for bacterial proliferation are prevailing. Given that seeds used for sprouting are non-sterile, human pathogens may propagate within sprouting modules. We performed artificial inoculation of organic fenugreek seeds with an *Escherichia coli* O157:H7 strain, and studied its proliferation and dispersal in two types of three-layered home sprouting modules. *E. coli* O157:H7 was introduced to either the top or bottom layer of modules, and its vertical dispersal was analyzed after 3.5 days of sprouts cultivation. Also composition of the indigenous culturable bacterial species of fenugreek seeds were determined by sequencing 16S rRNA genes of randomly selected isolates. Using a Beta-Poisson model we assessed the risks for consumer's exposure to *E. coli* O157:H7, if the bacteria is present on seeds in top or the bottom layer. We found that the inoculated strain dispersed both vertically up and down between the layers, and the risk for dispersal of *E. coli* is high in either direction, posing a high risk for consumer's exposure. *Enterobacter* and *Pantoea* were the most prominent genera on non-inoculated fenugreek seeds. To reduce the risk for dispersal of human pathogens and potential consumer's exposure, implementation of high hygiene standards is needed for both seeds and modules used for home sprouting of seeds.

Keywords: Beta-Poisson, dispersal, *E. coli* O157:H7, fenugreek, foodborne, sprouting, 16S rRNA

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P7

Microbiological safety of organic and conventionally grown berries

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The objective of this work was to assess the nutritional quality and potential microbiological safety aspects of organic berries (strawberries; raspberries, black/red currant) and compare with conventionally grown berries in Finland, Sweden and Norway. Culture based microbiological analysis revealed only small differences between organic and conventional berries when coliforms, total bacteria and fungi were analysed. Coliforms were in many cases below the level of detection - an exception being a single farm where both organic and conventional black currants have clearly higher levels of coliforms (up to 10^5 CFU/g). Moulds were typically at the level of 10^3-10^5 CFU/g representing genera such as *Cladosporium* spp., *Penicillium* spp., *Alternaria* spp., *Botrytis* spp. Based on culture based studies organic and conventional berries are equally microbiologically safe. The DNA based analysis did not indicate that either the fungal or bacterial microbiota differed based on cultivation method. There were some trends in higher or lower relative values for some taxa, but the overall composition did not seem to be different. It has to be noted that these studies were only performed in Nordic countries and that the results on the differences between organic and conventional berries may not apply to other geographical areas.

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