Ghent, September 20th 2020

Report of Short Term Scientific Missions (STSMs)

by researchers in the period 2017-2019

within HUPLANT COST Action CA16110

"Human Pathogenic Microorganisms in Plant Production Systems"

What are STSMs?

The EU COST Action supports exchange visits between researchers involved in a COST-Action. Scientists can visit an institution or laboratory in another country. Short Term Scientific Missions (STSMs) are exchange visits aimed at fostering collaboration, sharing new techniques and infrastructure that may not be available in other participants' institutions or laboratories. STSM are intended especially for young researchers. For more information refer to: www.cost.eu/COST_Actions/networking.

Who can apply?

STSMs are open for PhDs, PostDocs, and advanced career researchers employed at institutions in countries participating in our HUPLANT COST Action CA16110 "Human Pathogenic Microorganisms in Plant Production Systems", or at approved institutions.

In the past 2 years the HUPLANT COST Action has supported various researchers' individual moblity and this opportunity has been used to strengthen existing networks and foster new collaboration within the COST network.

Below you can find a brief report of the experience of some of these research exchanges

A selection of STSM reports within the HUPLANT COST Action CA16110

1. STSM "Bistable expression of *Salmonella* effector proteins in plant cells".

04/02/2019 – 01/03/2019

Salmonella enterica serovar Typhimurium is a human enteric pathogen that has the ability to multiply and survive endophytically in plants. Plants are considered as alternative host for Salmonella and fruits and vegetables as an important source of food-borne disease. Although there is not much about how Salmonella interacts with the plant, Salmonella Pathogenicity Island 1 (SPI-1) is one of the major molecular mechanisms required (Schikora et al., 2011). SPI-1 is a gene cluster that encodes a type III secretion system (T3SS) and effectors involved in epithelial cell invasion. SPI-1 undergoes bistable expression that leads to the differentiation of the population into SPI-1ON and SPI-1OFF subpopulations. This process has been shown to take place within the mouse gut and under

laboratory conditions and it has been shown to provide Salmonella with adaptive advantages that has been modelled as «cooperative virulence» (Sánchez-Romero and Casadesús 2018). However, there is no evidence of whether this process happens during plant colonization or in determining adaptation to plants. The EU Cost Action CA16110 gave me the opportunity to conduct a STSM in the laboratory of Adam Schikora, which allowed me to learn the proper techniques to work with Salmonella during its interaction with plants. We analysed a Salmonella enterica serovar Typhimurium strain carrying a chromosome-located transcriptional fusion to gfp of SPI-1 T3SS effector gene sipB in specific growing conditions, which are specific to the plant host. The experience gained during the STSM and other subsequent experiments have alowed us to identify bistable expression of Salmonella effector proteins during the plant colonization. As this situation involve an adaptative advantage during the colonizaton of animal tissue, we think that it is also relevant for Salmonella plant colonization.

Research exchange situated within HUPLANT COST Action CA16110 WG1. Ecology of HPMO in plants and in environments relevant for plant production

Report completed by Nieves López Pagán. Department of Cellular Biology, Physiology and Genetic. University of Malaga.

2. STSM "Assessment of the risk caused by persistence of human pathogens in soil"

11/12/2017 to 18/03/2018

During the stay at the Julius Kuhn Institut, Institute for epidemiology and pathogen diagnostics in Braunschweig, Germany an experiment was carried to determine the persistence of Salmonella enterica ser Typhimurium and Senftenberg in soil with and without sewage sludge compost amendment. To this end an experimental setup consisted of: 1) Mixing the sewage sludge compost with the soil 30 days prior to planting; and 2) Inoculation of those mixtures with Salmonella enteric to simulate soil contamination during fertilization. During this time soil samples were taken to determine starting Salmonella CFU counts and to extract total bacterial community DNA. After 30 days of soil incubation with the sewage sludge compost, 800 plants of Chinese cabbage (Brassica rapa L.) were planted. An additional experiment was performed simulating the contamination of soil with Salmonella enterica via irrigation water. Also, at this time point soil samples were taken to determine Salmonella enterica CFU counts and to extract total bacterial community DNA. Further sampling took place at 7, 21, 35 and 56 days post planting. Throughout the experiment, the upper plant tissues (phylosphere) were sampled to check for Salmonella enterica presence. To gain knowledge on possible Salmonella enterica internalization in plant roots and transfer pathways to upper plant tissues we used Confocal Laser Scanning Microscope. Chinese cabbage was grown in soil for 3 weeks and transferred to falcon tubes containing a solution with GFP-tagged

Salmonella enterica. To evaluate the interaction between Salmonella enterica and the native microbiome Denaturating Gradient Gel Electrophoresis (DGGE) was performed on the total bacterial community DNA. The 16S rRNA gene fragment was amplified using GC PCR and subsequently an electrophoresis process was carried out overnight. The 16S rRNA gene fragment from total community DNA was also sent for sequencing by Illumina MiSeq.

Research exchange situated within HUPLANT COST Action CA16110 WG1. Ecology of HPMO in plants and in environments relevant for plant production

Report completed by Nikola Major, Institute of Agriculture and Tourism, Poreč, Croatia.

3. STSM "Fresh produce as host for IncP-1 plasmids"

22/10/2018 to 20/11/2018

During this STSM, a technique called epicPCR (Emulsion, Paired Isolation and Concatenation PCR) was learned at the hosting institution i.e. the Department of Microbiology, University of Helsinki, Finland. This method allows to link the phylogeny and functional genetics of bacterial populations. The major purpose was to train epicPCR and being capable of performing the experiment self-contained. Now, the method can be established in the home institution for further studies. Apart from acquiring and training the methodological skills, the method was used the first time for the identification of bacterial populations from lettuce and tomato plants that carry MGEs such as IncP-1 plasmids or class 1 integrons. During the STSM, the experiment could be carried out successfully for intI1, which is the class 1 integron integrase gene, in lettuce and tomato. Class 1 integrons play a major role in the worldwide problem of antibiotic resistance as they can capture and express multiple resistance genes and, if located on plasmids or transposons, transfer those to other bacteria, including human pathogens. However, the initial goal of identifying IncP-1 plasmids and its hosts in fresh produce could not be reached, as the designed primers were not working successfully in epicPCR. In the future, it is planned to develop primers for epicPCR targeting the trfA gene of IncP-1 plasmids. After the four weeks in the hosting institution, samples were prepared and send to Illumina sequencing. Now, it remains to analyse the sequencing results and to finally identify which phyla in lettuce and tomato are the hosts of class 1 integrons. This analysis will be made in collaboration with the hosting institution at the university of Helsinki. The possibility to link microbial functions to the phylogeny of bacterial groups on fresh produce using epicPCR, which is the method learned during this STSM, can help to taxonomically identify human pathogenic microorganisms from plants. As fresh produce and its associated microorganisms are potentially consumed raw and can end up in the gastro-intestinal tract of humans, there is a risk of taking up not only commensals but also human pathogens. By using epicPCR, the bacterial hosts of mobile genetic elements (MGEs) can be identified and possibly a risk estimation of dissemination of transferable genes such as genes

promoting pathogenicity or antibiotic resistance can be made. This could contribute to improve agricultural practices and sanitary measures for the control of human pathogens. We hope to share the results obtained during the STSM as short communication in a peer-reviewed journal fulfilling the dissemination requirement of WG5 of the COST Action.

Research exchange situated within HUPLANT COST Action CA16110 WG2 "Taxonomical relatedness of HPMO with plant-associated microorganisms" and WG4 "Agricultural practices and sanitary measures undertaken for the control of HPMO in APS".

Report completed by Kristin Hauschild Julius Kühn-Institute, Federal Research Institute for Cultivated Plants (JKI) Institute of Epidemiology and Pathogen Diagnostics Braunschweig, Germany.

4. STSM „Targeting microbial populations using Next generation sequencing"

2019/02/01 – 2019/03/31

This STSM contributed to working group 2, which aims to improve and intensify the taxonomical identification of individual members of the plant microbiomes that are anticipated to pose a negative impact on human health. The STSM was hosted by Prof. Holger Daims and Dr. Petra Pjevac at the University of Vienna, Department of Microbiology and Ecosystem Science, Division of Microbial Ecology. The aim of the STSM was to learn and apply every step in the identification and quantification of the microbial population using Next-generation sequencing (NGS). Comprehensive microbiome analysis based on 16S ribosomal RNA (rRNA) gene sequencing approach targeting the bacterial and parts of archaeal diversity was made on soil and water samples collected for preliminary research in the frame of my PhD research. The main focus was on bacteria from class Gammaproteobacteria (Enterobacteriales) and phylum Firmicutes (Bacilli) which present bacteria that are human pathogenic micro-organisms (HPMO) in the agricultural production system. During the STSM I improved my knowledge in different methods of DNA extraction, tested different specific primers for polymerase chain reaction (PCR) and learn the method of quantitative real-time polymerase chain reaction (qPCR). I have also learned how to analyse data after sequencing using different software and custom scripts and pipelines established at the host institute.

This STSM helped me to improve and expand my knowledge in microbial research and it was of great interest and benefit for my future scientific development. The COST action made this collaboration possible and both parties have started to work closely together with further development of the DNA based techniques. We expect a joint publication emerging within this scientific collaboration.

Research exchange situated within HUPLANT COST Action CA16110 WG2. Taxonomical relatedness of HPMO with plant-associated micro-organisms.

Report completed by Katarina Kajan, Ruđer Bošković Institute, Zagreb, Croatia

5. STSM "The exo-proteome analysis of selected Bacillus cereus group strains"

10/08/2019 - 31/08/2019

During the STSM the applicant was introduced to the laboratory and involved in the selection of Bacillus cereus isolates for preparation of exudate proteins for ORBITRAP analysis and preparation of DNA from isolates for sequencing by the Nextseq and Nanopore sequencing.

The ORBITRAP analysis of the eight Bacillus cereus group bacteria evidently showed that the developed method was very useful for the analysis of the exo-proteome. The analysis showed the presence of hundreds of different peptides in the exo-proteome. A number of these could be affiliated with known toxins often present in the exo-proteome of B. cereus bacteria, several others were affiliated to peptides with hypothetical or unknown function. The products of some genes hypothesized to be bipartite toxins were identified in the exo-proteome of two the analyzed bacteria. Analysis of the "full" exo-proteome showed that the analysis can differentiate by the different strains and group them in a phylogenetically meaningful way.

The annotations of the genomes obtained by the Nextseq and Nanopore sequencing were used in the analysis of the ORBITRAP data. The isolated exo-proteome of the eight strais still need to be analyzed by more ORBITRAP runs and by more bioinformatics analysis, however the already obtained results and experiences show that ORBITRAP analysis of the exo-proteome of B. cereus group bacteria will expand our knowledge about toxins involved in gastro-intestinal diseases.

This STSM strengthened the collaboration between the Faculty of Technology, University of Novi Sad, Serbia and Department of Environmental Science, Aarhus University, Roskilde, Denmark. Furthermore, scientific issues proposed in STSM give significant inputs and open doors for the future collaboration between the researchers institutions and participation in international research projects. Another added value expected from the research collaboration will be the possibility to publish joint research papers, and present the results of research at scientific conferences.

Research exchange situated within HUPLANT COST Action CA16110 WG2 'Taxonomical relatedness of HPMO with plant-associated micro-organisms' and WG3 'Public health issues related to occurrence of human pathogens in plant environments'.

Report completed by Ružica Tomičić, Faculty of Technology, University of Novi Sad, Republic of Serbia

6. STSM "Whole genome sequencing and closing of the genome of selected Bacillus cereus group strains"

10/08/2019 - 31/08/2019

During this STSM the applicant was involved in selection of Bacillus cereus isolates for the preparation of DNA for whole genome sequencing by the use of Illumina NextSeq and Nanopore sequencing as well as proteomic analysis of the same isolates by the use of Orbitrap MS. High molecular weight DNA of sufficient quantity (above 50ng/ul) and quantity (260/230 and 260/280 ratios) was obtained from all eight strains of the analysis. The sequencing libraries prepared to be used with the Oxford Nanopore Technologies MinION platform yielded 15 Gigabases worth of data, corresponding to approximately 2Gigabases per sample or 400x sequencing depth. These reads were basecalled (guppy) and trimmed for qualities and adapters using the appropriate software (porechop) before using them for denovo assemblies. The denovo assemblies were either utilizing only the Nanopore reads (using the flye assembler) and then polished using the Illumina reads, or both types of reads were used at the same time for hybrid assemblies (unicycler). Complete, or nearly complete genomes, accompanied by complete plasmids were obtained from all strains. The nearly complete genomes are still being worked on in an effort to close them as well. The complete genomes and plasmids were then annotated (prokka) and are awaiting further analysis that will lead to publications. The annotations were used in the analysis of the ORBITRAP data. This STSM was significantly added to the collaboration between the Institute of Food Technology, University of Novi Sad, Serbia and Department of Environmental Science, Aarhus University, Roskilde, Denmark by sharing and learning the applicant new techniques and within the topics of the cost action, and together develop new knowledge on the genomes of an important group of potential pathogenic bacteria isolated from agricultural plant growing systems.

Research exchange situated within HUPLANT COST Action CA16110 WG2 'Taxonomical relatedness of HPMO with plant-associated micro-organisms' and WG3 'Public health issues related to occurrence of human pathogens in plant environments'.

Report completed by Zorica Tomičić, Institute of Food Technology, University of Novi Sad, Republic of Serbia

7. STSM "Immunity suppressive action of *Salmonella* effector proteins in plant cells"

01/02/2018 – 22/2/2018

The expression and translocation of effector proteins is a fundamental characteristic of biotrophic interactions and is required for pathogen virulence. The intention of this work was to investigate the mechanism used by human pathogens to deactivate the plant

immune system and to colonize the plant hosts. Knowledge on the possible way(s) of infection could lead to new strategies to protect plants, hence to protect human health. Overall, results and knowledge that will be obtained during this STMS at the host institution (the department of genetics, faculty of biology, university of Sevilla) will allow new input in the design of means to reduce the possibility of disease outbreaks related to plant products. During this STSM skills and knowledge have been acquired on defined protocols. For example, how to grow Salmonella under specific conditions, which induce the expression of SPI-1 or SPI-2 or how to test the expression of effector proteins encoded on either pathogenicity islands. To this end, modified strains with effector proteins, tagged with the catalytic domain of CyaA from Bordetella pertussis were used for the subsequent detection assay. CyaA is a calmodulin-dependent adenylate cyclase, which produces cAMP in the presence of Ca2+ and calmodulin (present only in eukaryotic cells). The following detection of an increase in cAMP concentration suggests the translocation of the tagged effector protein into host cell. The focus of this specific STSM action enabled me to establish experimental conditions allowing induction and detection of these tagged proteins in plant extract media as well as to assess their translocation into the plant cells at home institute. This STSM brought profits on multiple levels. Such as, acquisition of techniques how to specifically induce the expression of SPI-1 or SPI-2 encoded proteins, obtained knowledge on additional molecular techniques related to this human pathogen, new techniques of western blotting and ELISA and knowledge on the mechanisms of detection of fusion-proteins using in vitro and in vivo assays. Results obtained during the STSM will be part of my PhD thesis and should be shared as short communication in peer-reviewed journal fulfilling the dissemination requirement of WG5 of the COST Action. In addition, the acquired skills should be established at the home institute.

Research exchange situated within HUPLANT COST Action CA16110 WG1. Ecology of HPMO in plants and in environments relevant for plant production and WG4 "Agricultural practices and sanitary measures undertaken for the control of HPMO in APS".

Report completed by Azhar Zarkani, Julius Kühn-Institut, Federal Research Centre for Cultivated Plants (JKI), Institute for Epidemiology and Pathogen Diagnostics, Germany

8 STSM "Determination of potential human pathogenic bacteria from soil and crop plants on flooded regions and their pathogenicity"

26/08/2019 to 11/09/2019

Purpose of the STSM was to precisely identify strains of potentially pathogenic bacteria isolated from arable land and from vegetables in flooded areas. Untreated sewage water of all cities in BIH is poured directly into rivers. On this way, during the flood, these wastewaters came directly to the arable lands. We took samples of soil and vegetables from the affected area and isolated potentially pathogenic bacteria. For precisely identification of selected strains PCR method was used. Another purpose of STSM was to

test the pathogenicity of selected strains in experimental rats whether they retained their pathogenic properties since they were located in ecological niches that were not their natural habitat.

Isolation of potential human pathogen bacteria by indirect breeding methods was performed at home institution (University of Banja Luka). In Laboratory of Pharmaceutical Microbiology and Immunology (University of Belgrade) total DNA extraction and identifying the strains by PCR method were performed. Testing of their pathogenicity in experimental Wistar rats was performed at vivarium and laboratory for working with experimental animals. Except collecting the samples and isolation of potential pathogens all phases of the experiment were performed by applicant Svjetlana Lolić and mentor Jelena Antić Stanković. The first phase of experiment was performed by team from Faculty of Sciences in Banja Luka (4 members).

Achieved results show that floods are one of the ways of distribution of human pathogenic bacteria to the crops and arable land. Using PCR analysis, we confirmed the presence of four different pathogenic bacteria on vegetables and two in soil. Despite the fact that they were located in areas that were not their natural habitat, isolated strains of Escherichia coli and Salmonella enterica kept their pathogenic properties. The presence of the following bacteria on the vegetables was confirmed by PCR analysis:

- on lettuce leaves: Escherichia coli, Listeria monocytogenes, Citrobacter freundii
- on spinach leaves: Escherichia coli, Listeria monocytogenes
- on tomato fruit: Escherichia coli, Salmonella enterica, Citrobacter freundii
- on bell pepper: Escherichia coli, Salmonella enterica
- at the root of red onion: Salmonella enterica

In soil samples the presence of Salmonella enterica and Escherichia coli was also confirmed by PCR analysis.

Isolated strains of *E. coli* were O26 H11 type which is enterohaemorrhagic and has ability to cause diarrhoea and the haemolytic uraemic syndrome (HUS). Confirmed isolated strains of Escherichia coli and Salmonella enterica were used for testing their pathogenicity in experimental Wistar rats. The average leukocyte count and lymphocyte content in the differential blood count in both groups of infected animals statistically significant increased. The proportion of neutrophils in the blood of all three groups did not change significantly, and percentage of monocytes, basophils and eosinophils were lower than in control group. Experiment showed that infection with Escherichia coli leads to decreasing values of the erithrogram. The changes in these hematological parameters may be associated with hemolytic effect that causes Escherichia infection, which is reflected in reduced values of red blood cell parameters. Comparison of red blood count of healthy individuals with

Salmonella infected specimens showed that there is no statistically significant difference for any red blood cell parameter.

We are currently writing a paper with the results achieved

Research exchange situated within HUPLANT COST Action CA16110 WG1. Ecology of HPMO in plants and in environments relevant for plant production and WG3. Public health issues related to occurrence of human pathogens in plant environments.

Report completed by Svjetlana Lolić, Faculty of Science and Mathematics, University of Banja Luka Bosnia and Herzegovina

Report compiled by Isabelle Virlogeux-payant (INRA, Fr), Mieke Uyttendaele (UGent, Be) and Diogo Proenca (UCoimbra, Pt) . The contribution of all researchers mentioned above that received an STSM Grant from HUPLANT COST Action CA16110 "Human Pathogenic Microorganisms in Plant Production Systems" in the period 2017-2019 is much appreciated.
Two researchers who received an STSM Grant in that period (Athanasios Servaz on parental leave and Aoife Joyce had not (yet)) provided their input and thus could not be included in this compilation report for HUPLANT COST Action CA16110 public website.