Euphresco

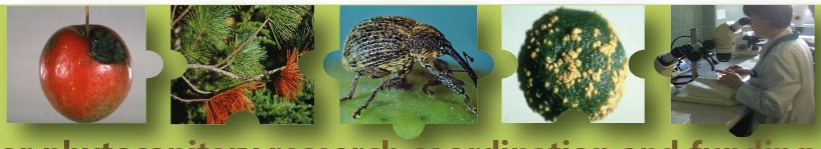
Final Report

Project title (Acronym)
Assessment of *Dickeya* and *Pectobacterium* spp. on vegetables and ornamentals (Soft rot)

Project duration:

Start date:	2015-01-01
End date:	2018-12-31

Contents



1. Research consortium partners

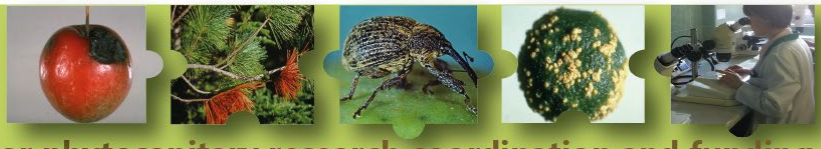
Coordinator – Partner 1			
Organisation	Wageningen University & Research (WUR)		
Name of Contact (incl. Title)	Jan van der Wolf	**Gender**:	M
Job Title	Bacteriologist, Senior Scientist		
Postal Address	P.O. Box 16, 6700 AA Wageningen, the Netherlands		
E-mail	Jan.vanderWolf@wur.nl		
Phone	+316 10028194		

Partner 2			
Organisation	National Plant Protection Organization, Netherlands Food and Consumer Products Safety Authority (NVWA)		
Name of contact (incl. Title)	Dr Maria Bergsma-Vlami	Gender	F
Job Title	Bacteriologist, head of the bacteriology team		
(Postal) address	Geertjesweg 15, Wageningen, The Netherlands		
E-mail	m.vlami@nvwa.nl		
Phone	+316 15891907		

Partner 3			
Organisation	Science and Advice for Scottish Agriculture (SASA)		
Name of contact (incl. Title)	Prof. dr Gerry Saddler	**Gender**:	M
Job Title	Bacteriologist, Director SASA		
Postal address	SASA, Roddinglaw Road, Edinburgh, Scotland, UK		
E-mail	Gerry.saddler@sasa.gsi.gov.uk		
Phone	+44 (0)1312448925		

Partner 4			
Organisation	French Association of seed potato producers (FN3PT-RD3PT)		
Name of contact (incl. Title)	Dr Valérie Hélias	**Gender**:	F
Job Title	Phytopathologist, Researcher, Group leader		
Postal address	UMR 1349 IGEPP INRA - Agrocampus Ouest Rennes - Université Rennes 1 BP 35327 F-35653 LE RHEU FRANCE		
E-mail	Valerie.helias@fnpppt.fr		
Phone	+33 (0)223485186		

Partner 5	
Organisation	ARO - Volcani Center (ARO)



Name of contact (incl. Title)	Dr. Leah Tsror; Dr Iris Yedida	**Gender**:	F; F
Job Title	Senior Scientist, Potato Disease expert; Bacteriologist, Senior Scientist		
Postal address	Department of Plant Diseases and Weed Research, ARO (Volcani), Gilat Research Center, M.P. Negev, 85250, Israel; The Institute of Plant Sciences, Ornamental Plants and Agricultural Biotechnology Dep. ARO, The Volcani Center, POB 15159 Hamakabim Road 68, 7528809 Rishon Lezion, Israel		
E-mail	tsror@volcani.agri.gov.il ; irisy@volcani.agri.gov.il		
Phone	+972 (0)89928660; +97239683387		

Partner 6			
Organisation	University of Helsinki (UH)		
Name of contact (incl. Title)	Dr Minna Pirhonen	**Gender**:	F
Job Title	Bacteriologist, University lecturer		
Postal address	Department of Agricultural Sciences, PO Box 27, 00014 University of Helsinki, Finland		
E-mail	minna.pirhonen@helsinki.fi		
Phone	+358 504922077		

Partner 7			
Organisation	Natural Resources Institute Finland (LUKE)		
Name of contact (incl. Title)	Dr. Yeshitila Degefu	**Gender**:	M
Job Title	Bacteriologist, Senior Scientist		
Postal address	Paavo Havaksen tie 3, 90014 University of Oulu		
E-mail	Yeshitila.degefu@luke.fi		
Phone	+358 295326068		

Partner 8			
Organisation	Potato Research Institute (PETLA)		
Name of contact (incl. Title)	Jussi Tuomisto	**Gender**:	M
Job Title	Director PETLA		
Postal address	Potato Research Institute, Alapääntie 104, 61400 Ylistaro, Finland		
E-mail	jussi.tuomisto@petla.fi		
Phone	+358 404507200		

Partner 9			
Organisation	Intercollegiate Faculty of Biotechnology University of Gdansk & Medical University of Gdansk (UG)		
Job Title	Bacteriologist, University Professor		

Name of contact (incl. Title)	Prof. Dr Ewa Lojkowska	**Gender**:	F
Postal address	Abrahama str. 58, 80-307 Gdansk, Poland		
E-mail	Ewa.lojkowska@biotech.ug.edu.pl		
Phone	+ 48 725991070		

Partner 10			
Organisation	Canadian Food Inspection Agency (CFIA)		
Name of contact (incl. Title)	Dr Sean Li	**Gender**:	M
Job Title	Bacteriologist, Senior Scientist		
Postal address	93 Mount Edward Road, Charlottetown, PE, Canada, C1A5T1		
E-mail	Sean.li@inspection.gc.ca		
Phone	+1 9023680950 ext. 263		

2. Short project report

2.1. Executive Summary

Dickeya and *Pectobacterium* belonging to the group of soft rot *Pectobacteriaceae* (SRP) are causing emerging problems in a wide range of vegetable and ornamental crops in Europe, including potato, carrot, cabbage, Chinese cabbage, celery, leek, pepper, parsley, *Zantedeschia,* hyacinth, *Dahlia*, *Chrysanthemum, Philodendron, Freesia, Saintpaulia, Iris, Aglaonema, Crocus, Campanula* and *Phalaenopsis*. The phytopathogens in both genera are genetically and phenotypically highly diverse. Disease problems in the different hosts are associated with the introduction of new variants or by spread of groups already present in Europe. Within this Euphresco project we aimed to identify and assess the risks of these new variants, and to develop management strategies, including reliable diagnostic methods to prevent introductions and further spread of SRP. To reach our goals, meetings were organized and collaborations were established with specialists worldwide. All information on meetings, protocols and activities of the Euphresco group are published on the Dickeya/Pectobacterium website, conveyed by the James Hutton Institute in Invergowrie (Scotland) (https://engage.hutton.ac.uk, contact person Dr I. Toth/Dr J. Fairly).

During the project, 1.5 days meetings were held in 2015 in Gdansk (Poland), in 2016 in Helsinki (Finland), in 2017 in Edinburgh (Scotland) and in 2018 in Emmeloord (The Netherlands). Meetings were attended by an average of 30 participants from organizations in EU member states, North- and Latin America, Africa, Asia and Australia.

One project's objective was to develop methods for the detection and identification of *Pectobacterium* and *Dickeya* species in different matrices. For this, a panel of reference strains has been compiled for *Dickeya* and *Pectobacterium* species. Most strains have been deposited in international collections. For most strains also whole genome sequence data are available. During the course of this project, several diagnostic tests were developed and evaluated, often based on the TaqMan technology.

In several countries, surveys in potato and ornamental crops were conducted, but also in other matrices of the potato ecosystem, including water used for irrigation. In addition, new taxonomic groups that have been identified, are now new species including: *P. versatile, P. aquaticum, P. fontis* and *P. polonicum*. In potato, *P. brasiliense* became dominant as blackleg causing organism and has largely outcompeted *D. solani* in the last five years. In surface water in Europe, *D. zeae* was found to be the dominant SRP. In other continents, serious outbreaks of potato blackleg with other SRP has been reported, such as *D. dianthicola* in the USA and Australia. Various new SRP have been described, namely *P. punjabense*, *P. peruviense*, *P. polaris*, *D. lacustris* and *D. zantedeschia*. For the first time, *D. fangzhongdai* was described in Phalaenopsis. Not all species can cause potato blackleg.

Studies on various virulence factors were conducted for SRP, such as on chemoreceptors, small phenolic plant compounds interacting with signal molecules of *Pectobacterium* involved in the quorum sensing mechanism of the pathogen. A Tn-seq approach was developed and used to identify new virulence factors.

Information was exchanged on disease management strategies which include cultivation practices, resistance breeding, hygiene and the use of (bio-) control agents. A strict hygiene and an intensive monitoring of seed lots was found to be associated with a significant reduction of infections with blackleg causing SRP. A phage therapy has been developed to protect (seed) potato tubers against soft rot during storage. Various bacteriophages and bacterial antagonists were characterized and some evaluated for control of potato soft rot and/or blackleg. Steam treatments for seed tubers were found to decrease the blackleg incidence. Cold plasma treatment was found to kill SRP grown *in vitro*. Similarly, stabilized silver nanostructures killed SRP. It was found that seed potato lots can differ in suppressiveness against *D. solani*. Indications were found that the microbiome in tuber tissue plays a role in this.

2.2. Project aims

Given the range of current or emerging *Dickeya* and *Pectobacterium* problems, there was a need to optimise resources by ensuring that research is well coordinated and is not duplicated nationally and internationally. The consortium searched for opportunities to cluster existing work to ensure added value through joined meetings, appropriate joint outputs and inclusion of appropriate additional work.

The overall goal of the project 2015-D-135 'Assessment of *Dickeya* and *Pectobacterium* spp. on vegetables and ornamentals (Soft rot)' was to identify and assess the risks of new variants of soft rot *Pectobacteriaceae* and new diseases caused by SRP, and to develop management strategies, including reliable diagnostic methods to prevent introductions and the further spread of SRP.

The project had the following objectives:

Diagnostic methods. To develop and identify reliable and cost-effective methods for the detection and identification of *Pectobacterium* and *Dickeya* species in different matrices. Development of protocols has partly been achieved within previously funded Euphresco projects on *Dickeya* and *Pectobacterium*[1].

Risk assessment. To assess the presence of different *Dickeya* and *Pectobacterium* species in vegetable and ornamental crops and in environmental/industry sources such as machinery, irrigation water as part of the risk assessment. For these surveys, a representative panel of SRP strains were compiled. Collected strains were characterised using molecular and phenotypic methods. Risk assessment also involved generating new information on the ecology of SRP in different hosts, including knowledge on dissemination and infection pathways, aggressiveness, survival strategies and plant pathogen interactions.

Disease management. To increase knowledge on disease management strategies via exchange of information, which includes information on cultivation practices, resistance breeding, hygiene and the use of (bio-) control agents.

[1] *Dickeya* species in potato and management strategies https://zenodo.org/record/1326445#.Xg8MnUdKjct
Assessment of *Dickeya* and *Pectobacterium* spp. on potatoes and ornamentals https://zenodo.org/record/1326504#.Xg8Mx0dKjct

2.3. Description of the main activities and results

The references refer to the abstracts published during the various meetings, see Annex I, II, III, IV

2.3.1. Meetings

The Euphresco consortium on SRP had been successfully collaborating since 2013. During the Euphresco project 2015-D-135, 1.5 days meetings were held in 2015 in Gdansk, in 2016 in Helsinki, in 2017 in Edinburgh and in 2018 in Emmeloord. Meetings were attended by an average 30 participants from EU member states, North- and Latin America, Africa, Asia and Australia. The exchange of information between scientists, members from national plant protection organizations, breeders and policy makers was of great support in finding novel strategies for disease management. The consortium agreed to continue as an informal group, which will be coordinated by Prof. Dr. R. Czajkowski (UGdansk). From 2019 onward, no new Euphresco project on SRP will be conducted.

2.3.2. Diagnostic methods

One goal was to develop and fine-tune reliable and cost-effective methods for the detection and identification of *Pectobacterium* and *Dickeya* species in different matrices. For this, a panel of reference strains was compiled for *Dickeya* and *Pectobacterium* species. The majority of the strains were deposited in international collections. For most strains also whole genome sequence data are available. The various diagnostic methods were extensively discussed including 1. isolation, 2. characterization of bacteria using multi-locus sequence analysis, 3. testing for virulence, 4. enrichment of SRP, 5. detection with TaqMan, LAMP tests and microarray techniques, 6. multiplex detection with the (Luminex) xTaq technology, and 7. High-Throughput Sequencing (HTS)-analysis in complex substrates. During the course of this project, several new diagnostic tests were developed and evaluated, often based on the TaqMan technology. For example, in the Netherlands new TaqMan tests were developed for *P. brasiliense* and *P. parmentieri* (Van der Wolf, 2015). In France, 11 real-time PCR tests were evaluated in comparative testing, including tests for *P. atrosepticum*, *Dickeya* spp., *D. solani*, *D. dianthicola*, *P. brasiliense* and *P. parmentieri* (Hélias, 2016). Pectinolytic bacteria isolated from blackleg diseased plants can be easily and rapidly identified by *gapA* sequencing (Cigna *et al*., 2017). *D. solani* can be easily identified using potato dextrose agar on which the pathogen grows as wrinkled and brownish colonies (Pirhonen, 2017). It was found that post-harvest seed lot testing can be reliably used to predict stand losses due to infections with *D. dianthicola* in the next growing season (Secor, 2016).

Unfortunately, a Test Performance Study (TPS) for detection of SRP in potato, to be organized by the Dutch National Plant Protection Organization (NVWA), had to be cancelled.

2.3.3. Surveys and risk assessments

Surveys in potato and ornamental crops were conducted in several countries, and the presence of SRP was also investigated in the potato ecosystem, including sources of irrigation water, using the diagnostic methods described previously. In Poland, *P. parmentieri* was dominant in diseased plants, but also infections with *P. atrosepticum*, *P. carotovorum*, *D. solani* and *D. dianthicola* were found (Lebecka *et al.,* 2015*).* In contrast, in surface water sampled in Poland, only *D. zeae* and *D. chrysanthemi* were found (Potrykus *et al*., 2015). In Norway, *P.*

carotovorum and *P. atrosepticum* were dominant in diseased plants whereas only in a single case infection with *P. parmentieri* and *D. solani* were found (Lebecka *et al*., 2015). In Switzerland, *P. brasiliense* was the most prevalent in blackleg symptomatic plants, followed by *P. carotovorum, P. parmentieri, D. dianthicola* and *D. solani* (Dupuis *et al*., 2017). In Finland a group of strains was isolated from potato samples which were similar to, but nevertheless differentiable from *P. polaris* and may represent a new species or subspecies of *P. polaris* (Pirhonen *et al*., 2018). In the Netherlands, *P. brasiliense* is dominant in blackleg diseased plants and symptomless infected seed tubers (Vreeburg, 2017). Strains isolated from diseased plants were mainly virulent, but non-virulent and hypovirulent strains were isolated from latently infected tubers. *P. brasiliense* was also detected in and isolated from insects. In comparative testings in Switzerland, *D. solani* was the most aggressive one, whereas strains of *P. carotovorum* did not induce blackleg symptoms. In Scotland, still *P. atrosepticum* is the main causative organism of potato blackleg, although incidentally *P. parmentieri* and *P. brasiliense* are detected (Saddler, 2015, Cahill, 2016). Infections occurred in Scotland already in the first field generation. In Israel, diseased plants were infected with *D. solani* and *P. brasiliense* (Tsror, 2015). In France, beside several *Pectobacterium* spp., *D. solani* and *D. dianthicola* were found in potato (Pedron *et al*., 2015; Hélias *et al*., 2016). The group of *D. solani* strains could be subdivided in two groups on the basis of single nucleotide polymorphism SNP's, indels and additive horizontal gene transfers. Several newly identified groups, that are now species, as *P. versatile* (Hélias *et al*., 2018) were found in culture collections; *P. punjabense* isolates were sporadically identified from French and Dutch laboratory collections (Cigna *et al*., 2018). A *P. punjabense* strain from Pakistan was able to cause blackleg. In Chile, *P. atrosepticum*, but likely also other SRP variants (*P. carotovorum* and *D. dadantii*) are responsible for high losses of up to 30% in potato production (Acuna *et al*., 2015, 2016, 2017). A soft rot causing strain isolated from potato in Peru was found to be a new species and was named *P. peruviense*. The same species was also isolated from alpine rivers in France. In the USA, blackleg is caused predominantly by *D. dianthicola*, which seem to originate from different sources as the isolates were diverse (Hao *et al*., 2016, 2018; Secor *et al*., 2017). In the USA, *Dickeya dianthicola* was found in potatoes in the stolon end, the sprout, but most frequently in the peel strip, indicating that testing of the stolon end is not sufficient (Secor *et al*., 2017). In Canada, *D. dianthicola*, *P. parmentieri*, *P. atrosepticum* and *P. carotovorum* are the most prevalent blackleg causing agents (Li, 2016). In South Africa, water samples from dams, boreholes, rivers and overhead irrigation systems in five potato growing areas were surveyed for *Pectobacterium* and *Dickeya* species. Identification was done by REP-PCR and Multiplex PCRs, using species specific primers. Initial tests revealed that several bacteria are detectable in water systems used for irrigation in most regions sampled, among which *P. brasiliense*, *P. atrosepticum*, *P. carotovorum* and *Dickeya* spp. (Laughton & Van der Waals, 2016; Van der Waals *et al*., 2016). A high variation of SRP are found in water ways in France, including a new Dickeya species named *D. lacustris* (Bertrand, 2017; Pedron *et al*., 2018; Hugouvieux-Cotte-Pattat *et al*., 2018). Introduction of virulent variants present in water ways may pose a threat to agriculture and horticulture. In 2017 in Australia, an outbreak of *Dickeya dianthicola* in (seed) potatoes was reported. Likely, infected Dahlia tubers were the initial infection source. The pathogen was also detected in freesia bulbs (Mann *et al*., 2017).

In Phalaenopsis grown in Slovenia, *Dickeya sp.* were isolated which were similar to *D. fangzhongdai* found in China, that causes a bleeding canker of pear (Alic *et al*., 2016). A soft

rot causing strain isolated from Calla lilly was found to be a new species and was named *P. zantedeschiae* (Waleron *et al*., 2018).

An important issue was the sudden changes in the population structure of SRP in potato in Europe. Between 2000 and 2010, *D. solani* was the most dominant causative agent of potato blackleg in the seed production in several countries on the European continent, but in the last 5 years *P. brasiliense* became increasingly prevalent in Switzerland and elsewhere (De Werra, 2015). Nevertheless, outbreaks as a result of infections with *D. solani* are still reported (Degefu, 2015). Research was conducted to find the driving force behind it. *P. brasiliense* may originate from alternative host plants, including weeds. In Israel, the pathogen was isolated from latently infected *Malva nicaeensis* plants (Tsror *et al*., 2019).

The population of *D. solani* strains in Europe is still highly clonal, although the intraspecific diversity is slowly growing (Pedron *et al*., 2017; Lojkowska *et al*., 2017; Van Gijsegem, 2018). The global population of *P. atrosepticum* strains, although phenotypically similar, showed a high intraspecific variation (Pritchard and Toth, 2017). Similarly, a high genetic variation was found between strains of *P. parmentieri* (Lojkowska *et al*., 2017, 2018).

The survival of *P. brasiliense* in soil was studied in pot trials under different temperature and moisture regimes (Van der Waals *et al*., 2015). Six weeks after inoculation, no culturable cells could be detected, although after 5 months positive reactions were still found in a real-time PCR test. In Scotland, it was shown that *P. atrosepticum* (a related pathogen) was able to move in a seed production field during the growing season from diseased to healthy plants (Campbell *et al*., 2015). Free-living nematodes were found able to disperse soft rot bacteria which may play a role in the observed spread of the pathogen in soil (Pirhonen *et al*., 2015).

During flailing (mechanical haulm destruction) of a SRP infected seed potato crop, contaminated aerosols could be easily trapped at a short distance from the crop with an air samplers, indicating aerosols as a potential (initial) infection source. Aerosols can be wind blown over long distances (up to 1 km) and potentially infect adjacent crops (Van der Wolf *et al*., 2018).

A natural solution to provide safe irrigation water is managed aquifer recharge (MAR) for agriculture. Here, tile drainage water (TDW) is collected and infiltrated in brackish aquifers, resulting in a fresh water ‘bubble’ in the subsurface which gives farmers access to sufficient fresh water to irrigate their crops even in times of drought. To prevent the occurrence of crop diseases, the removal of selected plant pathogens in TDW and during aquifer soil passage is required. Under aerobic conditions *D. solani* and *P. carotovorum* were not detected anymore in 0.1-ml samples within 14 days at 10 ºC. *D. solani* and *P. carotovorum* were no longer detected within 6 days at 25 ºC (Eisfeld *et al*., 2018)

Field experiments in the Netherlands using vacuum infiltrated tubers showed that *P. atrosepticum* and some strains of *P. brasiliense* were highly aggressive, *D. solani* and *P. parmentieri* were moderately aggressive, whereas four different *P. carotovorum* strains did not induce blackleg symptoms (Van der Wolf *et al*., 2015). This lack of virulence for *P. carotovorum* was also found in field experiments in Finland (Pasanen & Pirhonen, 2015)

2.3.4. Plant pathogen interactions

Within the frame of risk assessment, studies on molecular plant pathogen interactions were also conducted. Chemoreceptors were identified and the response to various compounds were studied (Hugovieux Pattat, 2016). Small phenolic plant compounds in *Pectobacterium* were found to influence biofilm formation via the quorum sensing mechanism (Joshi *et al*., 2015; Yedidia *et al*., 2016, 2017). External application of the signal molecules resulted in a full recovery of the infection capacity. In *P. brasiliense*, compounds were found that supported the pathogen to compete with other bacteria in planta (Moleleki, 2016). *P. atrosepticum* was localized as emboli in xylem vessels, multicellular structures of potato plant and bacterial compounds, to protect the bacteria from recognition by the defence mechanism of the host (Gorshkov *et al*., 2017). The production of coronafacic acid is important in the transition from the 'amenable' to the 'devastating' mode for *P. atrosepticum* in xylem vessels. For *P. atrosepticum* also adaptive proliferation was demonstrated that occurs under stress conditions, when the population density is too low to implement cell-to-cell communication. This proliferation is evidently supported by endogenous cell resources (Gorshkov *et al*., 2018). A transposon sequencing (Tn-seq) approach was developed and used to identify new virulence factors in *D. dadantii* (Royet *et al*., 2017).

The expression of virulence determinants of bacterial strains from different hosts is dependent on the tested plant species (i.e. *Brassica oleracea* or *Zantedeschia aethiopica*); whether it is a host or a non-host of the strain (Khadka and Yedidia, 2018). Differential gene expression was also demonstrated in the induced-resistance response of the plant hosts to the different bacterial strains.

It was found that repeated cultivation of strains of *D. dianthicola*, *D. solani* and *P. parmentieri* on agar media can result in a loss of virulence. To test for the blackleg causing capacity of strains or the susceptibility of cultivars field studies should be conducted; i*n vitro* maceration assay on potato tubers are not useful for this.

2.3.5. Disease management

In the Netherlands, an existing programme of strict hygiene and an intensive monitoring of seed lots resulted in a significant reduction of infections with blackleg causing SRP's (Kristelijn, 2015).

Broad host range bacteriophages able to infect the dominant *Pectobacterium* and *Dickeya* species were isolated after which they were genetically and phenotypically characterized (Czajkowski, 2015, 2016). The potential of bacteriophages was discussed. A phage therapy was developed in Scotland to protect (seed) potato tubers against soft rot during storage (Blackwell, 2017).

In France, 24 potato hybrids originating from three wild resistance donor species (*S. andigena*, *S. brevidens* and *S. etuberosum*) were tested for their susceptibility to soft rot (Hélias et al., 2016). Of these hybrids, 10 showed a very low severity against two out of five SRP species.

Several groups worked on the biocontrol of SRP's. For a *Pseudomonas* strain inhibiting SRP's *in vitro,* a hypothetical gene coding for an antimicrobial metabolite was knocked out after which the biocontrol strain lost its ability to inhibit the growth of *Dickeya* spp. *in vitro* (Krzyżanowska,

2015). Transcriptome profiling using a *Serratia plymuthica* biocontrol strain showed that treatment of potato tubers resulted in expression of genes involved in stress responses, hormone signalling and plant defence (Hadizadeh *et al*., 2016). In France, furrow application with *Pseudomonas putida* and *Rhodococcus erythropolis* biocontrol strains resulted in a reduction of the blackleg incidence and a decrease of seed infections (Munier *et al*., 2017). For the control of soft rot in potato during storage, compatible mixtures of antagonistic bacteria were successfully used (Krzyzanowska *et al*., 2018).

In Israel, seed tuber treatments were evaluated for their ability to reduce tuber rot during the fall-winter season, especially at early planting during September when temperatures are relatively high. The seed treatments included dry steam, oxolinic acid, mancozeb and MB5K (surface sterilizing agent) applied prior to planting. All treatments reduced seed decay or tuber rot, however, the results were inconsistent in field trials conducted in different years. Direct current atmospheric pressure glow discharge, also known as cold plasma, was shown to eradicate plant pathogenic bacteria, including *D. solani* (Motyka-Pomagruk *et al*., 2018). Silver nanostructures stabilised by pectin or sodium dodecyl sulfate (SDS) possessed strong antimicrobial properties against *Dickeya* and *Pectobacterium* and may be used in disease management programs (Motyka-Pomagruk *et al*., 2018).

Seed potato lots can differ in suppressiveness against *D. solani*. If seed lots from different origin with a low infection rate were vacuum infiltrated with high densities of the pathogen and planted in the same soil, remarkable differences in disease incidence were found (Van der Wolf *et al*., 2017). Indications were found that the microbiome in tuber tissue plays a role in the suppressiveness. Next studies are focussed on the manipulation of the microbiome.

Within the genus *Dickeya*, *D. solani* has been recognized as a threat, particularly for crops grown in hot climates. Furthermore, for many years *D. dianthicola* has been responsible for losses in various crops, under which potato. *D. dadantii* has been found in various ornamental crops and occasionally, *D. dadantii* occurs in potato in Europe. This raises questions about distribution and risks of this *Dickeya* species. In waterways in the UK and Finland *D. aquatica* and also *D. chrysanthemi* and *D. zeae* in Poland have been found, but the host has not been identified yet. There are also *Dickeya* variants not present in Europe yet, for which risks have not been assessed, such as *D. fangzhongdai* found in China, that causes a bleeding canker of pear.

Also, within the genus *Pectobacterium* (for Europe) new variants were described recently from which some have already a high impact. *P. brasiliense* and *P. parmentieri* (earlier wrongly classified as *P. carotovorum* subsp. *carotovorum*) are broad host range pathogens that cause emerging problems in potato in Europe. Similarly, *P. aroidearum* and *P. carotovorum* subsp. *odoriferum*, both with a wide host plant spectrum, are recognized to cause economic losses in ornamental plants, particularly in flower bulbs.

Given the range of current or emerging *Dickeya* and *Pectobacterium* problems, there is a need to optimise resources by ensuring that research is well coordinated and is not duplicated nationally and internationally. There are opportunities to cluster existing work to ensure added value through joined meetings, appropriate joint outputs and inclusion of appropriate additional work (*Dickeya* network and Euphresco consortium).

2.4. Conclusions and recommendations to policy makers

This project strongly contributed to a rapid and extensive information exchange on the characterization of (new) soft rot and blackleg causing pathogens, on virulence mechanism, on spread, survival and colonization and on the disease management via hygiene, cultivation measures and via treatments of plants for planting. It further resulted in the availability of improved diagnostic methods for detection and identification. The annual meetings stimulated collaborations of participants in research projects and publication of shared data.

The observation that the population structure of soft rot *Pectobacteriaceae* (SRP) rapidly change, in particular in potato is highly important. One example is the introduction of a subgroup of *Pectobacterium brasiliense*. This introduction may originate from contaminated insects, rain-water, surface water or from windblown aerosols, but contact infections with (debris of) plants grown in rotation with potato cannot be excluded. The finding and characterization of several new variants of (SRP) indicates that there is risk for introductions of new aggressive soft rot causing pathogens from the environment. It stresses the importance of regular surveys in order to keep diagnostic methods and other management tools methods updated.

The high risks for initial infections from environmental sources that can hardly be controlled, including rain-water, indicates the need for management practices that reduce the risk for spread and symptom expression. To reduce spread, the role of hygiene and cultivation methods are of utmost importance, such as reducing the risks for the presence of symptomatic plant material during harvest and post-harvest processes. So far, attempts to select cultivars with a high level of resistance against SRP failed. The use of improved selection and modern breeding techniques may be helpful to develop genotypes with a high, consistent level of resistance.

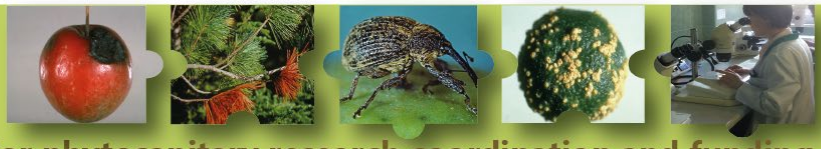
To limit damage, treatments of planting material are highly desirable. A combination of physical treatments to reduce inoculum levels of planting material followed by the application of biological agents (antagonists) able also to control the pathogen inside plants is a strategy that is expected to reduce inoculum load and symptom development. The improved knowledge on molecular plant pathogen interactions, such as the effect of phenolic compounds on the virulence of the pathogen may also result in the selection of cultivars or the development of agents that can reduce damage by SRP.

2.5. Benefits from trans-national cooperation

Given the range of current or emerging *Dickeya* and *Pectobacterium* problems, there was a need to optimise resources by ensuring that research is well coordinated and is not duplicated nationally and internationally. Via joined annual meetings, this Euphresco initiative strongly supported exchange of information and strains, discussions on disease management and control of soft rot diseases, and collaborations via research projects. Collaborations between members of this group are evidenced by several joined publications published during the course of the project.

The members of the group are highly motivated to meet annually also in the future. In June 2020, they will participate in the 4th International *Erwinia* Workshop, organized by Prof. Dr. Ian Toth (James Hutton Institute, Scotland) as a satellite meeting of the International Conference on Plant Pathogenic Bacteria in Assisi (Italy). From 2021 onward, the consortium will continue

to collaborate as an informal Dickeya/Pectobacterium group chaired by Prof. Dr. R. Czajkowski (Gdansk, Poland).



3. Publications

3.1. Article(s) for publication in the EPPO Bulletin

None.

3.2. Article for publication in the EPPO Reporting Service

None.

3.3. Article(s) for publication in other journals

- Chawki K., Quêtu-Laurent A.G. Taghouti G., Caullireau E., Fischer-Le Saux M., Le Hingrat Y., Andrivon D., Portier P. (2018). The Pectobacterium complex: Diversity and phylogeny. Phytopathology Volume 108, Number 10S : S1.30
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geographic location from water streams and symptoms on diverse plants. Int J Syst Evol Microbiol. Int J Syst Evol Microbiol 69:3214–3223. DOI 10.1099/ijsem.0.003611

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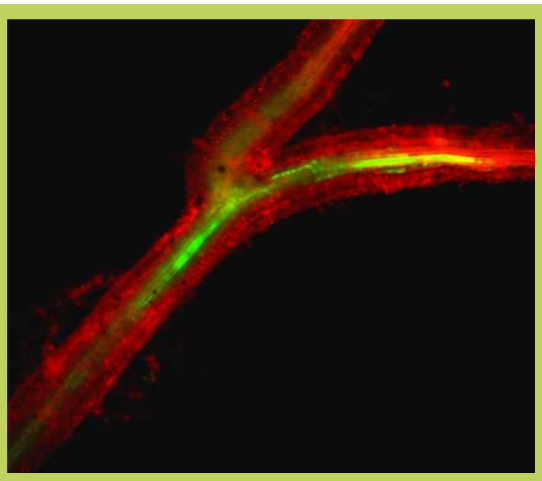
4. Open Euphresco data

None.

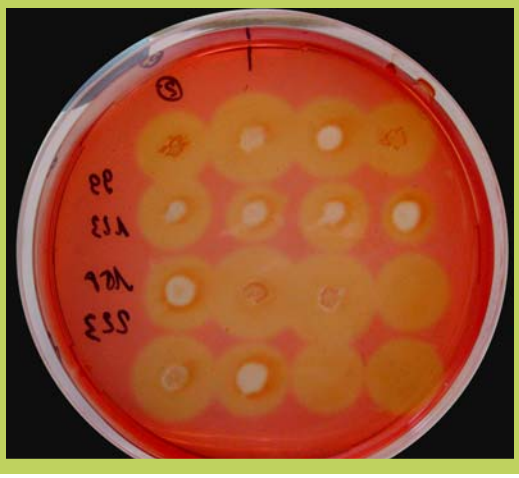
Euphresco

Euphresco III

“Dickeya & Pectobacterium meeting”








22 - 24 November 2015, Gdansk, Poland

Local organizer:
Prof. PhD E. Lojkowska (IFB UG & MUG, Gdansk, PL)
PhD Robert Czajkowski, PhD Marta Potrykus,
PhD Wojciech Sledz, PhD Małgorzata Waleron
Agata Motyka, Sabina Żołędowska

Project coordinators: **PhD Maria Bergsma (NVWA, NL) and PhD Jan van der Wolf (WUR, NL)**

The MOBI4Health project has received funding from the European Union's Seventh Framework Programme for reserarch, technological development and demonstration under grant agreement no 316094 and from the Ministry of Science and Higher Education

The effect of soil temperature and moisture on survival of *Pectobacterium carotovorum* subsp. *brasiliense* in fallow soil: Preliminary results

J.E. van der Waals, N. Laughton & L. Nhukarume

Department of Plant Science, University of Pretoria, Private Bag X20, Hatfield, Pretoria, 0028, South Africa

Monitoring of pathogen levels in soil is an important tool for disease management and understanding pathogen ecology. One of the most important pathogens in the South African potato industry is *Pectobacterium carotovorum* subsp. *brasiliense* (Pcb), the causal agent of soft rot and blackleg of potatoes. Although many articles have stated that pectinolytic bacteria are not able to survive for extended periods of time in soil in the absence of a host, none of these studies were conducted on Pcb. The aim of this experiment is thus to determine how long and under which environmental conditions Pcb is able to survive in fallow soil. Pilot pot trials using a clay-loam soil were set up at three different temperatures, 10, 20 and 30˚C. The soil in each of the temperature treatments was maintained at two different moisture conditions i) dry (no watering at all) and ii) wet (saturation point). A 108 cfu/ml suspension of a GFP-tagged Pcb isolate was used to inoculate the soil. Soil samples were taken regularly after inoculation from each temperature / moisture treatment to determine survival of the bacterium in the soil. A dilution series was made of each soil samples and plated out onto selective medium. Resultant colonies were counted and viewed under fluorescent light to confirm the presence of the GFP-tagged isolate. The concentration of bacteria in samples was determined also determined by qPCR. Preliminary results showed that four to six weeks after inoculation Pcb was no longer detectable in soil by conventional plating out. However, bacteria were still detected by qPCR up for five months after inoculation of the soil. This is probably due to amplification of non-living bacterial DNA in the soil. Pcb survived longest in the wet soil and at warmer temperatures than in dry soil or at 10˚C. This trial confirms results of various other studies on survival of pectinolytic bacteria, which indicate that these bacteria are unable to survive in soil for extended periods of time in the absence of a host. A second trial is currently underway to verify the results of the first.

Monitoring of imported and national seed lots in the control of pectinolytic bacteria in the Swiss potato Industry (2013-2015)

Patrice de Werra and Andreas Keiser

School of Agricultural, Forest and Food Sciences (HAFL), Bern University of Applied Sciences, Switzerland

During recent years, possibilities of minimizing the impact of blackleg in Swiss seed potato production have been discussed. Complementary to the official seed potato certification scheme based on visual inspection in the field, a reliable routine method to assess the health status of seed tuber lots is being sought.

In an on-farm project conducted on behalf of the Swiss Federation of Seed Potato Growers (swisssem) from 2013 to 2015, the relationship between seed infection and blackleg incidence in the field was investigated. PCR/qPCR tests were performed to detect latent infections in seed lots by the causal agent of blackleg. The focus was on Dickeya sp. (Dsp), P*ectobacterium atrosepticum* (Patr), *Pectobacterium wasabiae* (Pwas) and *Pectobacterium carotovorum* subsp. *brasiliense* (Pcbr), which are the most problematic pectinolytic bacteria encountered recently in Switzerland. Having been in steep decline in Switzerland since 2012, Dsp and Patr were almost absent in 2015, in both seed latent infection and in the field. Since 2013, Pcbr has gained in importance and was still the most problematic agent of blackleg this year.
Although the reliability and usefulness of PCR/qPCR tests as a diagnostic tool seem to be acceptable for Dsp and Patr, this is not the case for Pcbr. This bacterium was found in latent infections in a very large part of the seed lots analyzed, but was not consistently seen in the field. This led to an increased number of false positive analyses, which weakened the reliability of the test for this bacterium.

Understanding the epidemiology of *Pectobacterium* in-field spread and blackleg disease

Emma Campbell[1], Sonia Humphris[1], Greig Cahill[2], Yvonne Nova[2], Gerry Saddler[2], John Elphinstone[3], Stuart Wale[4] and Ian Toth[1]

[1]James Hutton Institute, Invergowrie, Dundee DD2 5DA
[2]Science and Advice for Scottish Agriculture (SASA), Edinburgh EH12 9FJ
[3]Fera Science Ltd., York YO41 1LZ
[4]Scotland's Rural College, (SRUC), Aberdeen AB21 9YA

Experimental plots were used to track the movement of a streptomycin resistant strain of *Pectobacterium atrosepticum* (Pba) from an infected central zone to healthy neighbouring plants. During the growing season, leaf, stem, root and tuber samples were taken from plants at radiating points from the central infector zone and tested for the presence of the marked strain and naturally occurring isolates. At the end of the growing season samples were taken from the stem of all plants showing blackleg symptoms and the pathogen present was identified. After haulm destruction the tubers designated to be sampled for assessment of infection were hand dug from the plot and tested to determine whether *Pectobacterium* infection was systemic (found in the vascular tissue of stolon end), or as lenticel infection in tuber peel. Our results confirmed movement of the streptomycin resistant Pba strain from infected to healthy plants. However, the data also revealed that natural isolates of *Pectobacterium*, including but not only Pba, were more prevalent than Pba 1039 strep^R in terms of both contamination and in causing blackleg disease. Pba 1039 strep^R and natural isolates of *Pectobacterium* were detected in both the peel and stolon end of the harvested tubers.

Lytic bacteriophages against soft rot Enterobacteriaceae – isolation, characterization and future perspective

Robert Czajkowski[1], Z. Ozymko[1], V. de Jager[2], E. Lojkowska[1],

[1] Department of Biotechnology, Intercollegiate Faculty of Biotechnology UG and MUG, Kladki 24, 80-822 Gdansk, Poland
[2] Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, the Netherlands

Pectobacterium spp. and *Dickeya* spp. are necrotrophic bacterial pathogens of many important crops worldwide. The effective strategies to control pectinolytic bacteria have not yet been developed. Consequently, the management of *Pectobacterium* spp. and *Dickeya* spp. is based mainly on the exclusion of infected plant material and the use of hygienic practices during potato cultivation and in storage. This study reports on the isolation and characterization of broad host lytic bacteriophages able to infect the dominant Pectobacterium spp. and Dickeya spp. affecting potato in Europe viz. *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc), *P. wasabiae* (Pwa) and *Dickeya solani* (Dso) with the objective to assess their potential as biological disease control agents. The lytic bacteriophages were isolated from potato samples collected in different potato fields in Poland. The phages were characterized for features that are potentially important for successful biological control applications and bacteriophages' stability in the environment. Transmission electron microscopy was used to study bacteriophage morphology. In the interaction studies, the phages were characterized for optimal multiplicity of infection, the rate of adsorption to the bacterial cells, the latent period and the burst size. The selected bacteriophages were also genotypically characterized with RAPD-PCR and RFLP techniques. The structural proteomes of selected phages were obtained by fractionation of phage proteins by SDS-PAGE. Phage protein identification was performed by liquid chromatography-mass spectrometry (LC-MS) analysis. Pulsed-field gel electrophoresis (PFGE), genome sequencing and comparative genome analysis were used to gain knowledge of the length, organization and function of the genomes.

Dickeya solani, the game changer in the 2015 cropping season potato blackleg outbreaks in north Finland

Yeshitila Degefu and Holappa, O.

Natural Resources Institute Finland (LUKE), Green Technology, PaavoHavaksen tie 3, FI-90014 University of Oulu, Finland

During the last three decades, the etiology of blackleg and soft rot in Finland has shifted from a serotype of one species - *Pectobacterium atrosepticum* to multiple species. To date *Pectobacterium atrosepticum*, *Pectobacterium carotovorum* subsp. *brasiliense*, *Pectobacterium carotovorum*, *Pectobacterium wasabiae* and *Dickeya solani* are frequently detected from potato with typical blackleg symptoms. Furthermore, it is intriguing to observe a significant number of samples with typical blackleg where none of these currently known potato blackleg and soft rot bacteria are detected. A circumstance tempting to suggest "unknowns" to the list and a revisit to the etiology of the disease complex. The *Dickeya* and *Pectobacterium* species listed above are known to be adapted to different temperature optimums and nowadays disease has become a likely phenomenon in Finland irrespective of the prevailing summer weather- warm or cool.

According to the 2015 weather statistics report by Finnish Meteorological Institute (http://en.ilmatieteenlaitos.fi/press-release/98978129) June was unusually cold 1-2 degrees colder than usual with exceptionally high precipitation especially in North Finland. July was exceptionally cold. The monthly average temperature in July remained below the long-term average in the whole country, varying from just over 15°C in southern parts of the country to less than 11°C in northern Lapland. A July as cool as this year occurs in the region on average once in 30 years. After two months of colder-than-average weather, August turned out to be warmer than average across the whole country. The average temperature for the whole country was 14.9°C, which was 1.4°C above normal levels. The month also saw a total of 15 hot days (where temperature rose over 25°C). The last time that August was the hottest month of the summer was in 2006.

Until the end of July, no blackleg symptoms were reported by farmers in the region. Just in the first and second week of August, severe and rapidly spreading blackleg outbreaks were reported from two separate fields of the same variety and seed background. The game changer was *D.solani*. Further characterization of the outbreaks and analysis of weather data will be discussed.

Effect of lenticular and vascular infection with Dickeya spp. on blackleg symptom expression in potato fields

Brice Dupuis, Gaétan Riot, Isabelle Kellenberger and Santiago Schaerer

Agroscope, Institute for Plant Production Sciences IPS, 1260 Nyon 1, Switzerland

Potato blackleg is known to be a seed borne disease caused by pectinolytic bacteria belonging to the genera *Pectobacterium* and *Dickeya*. The seed tuber can be infected through the lenticels and/or through the vascular system of the plant. Lenticelar inoculation is broadly used to assess the aggressiveness of *Pectobacterium* and *Dickeya* strains and is also commonly used to assess the susceptibility of the cultivars to blackleg. Nevertheless, little is known about the relevance of those results to assess the risk of transmission of the inoculum to the progeny tubers, through the vascular system of the plant. Field experiments were set up in Switzerland to answer this question: 4 to 19 cultivars were inoculated with one *D. dianthicola* strain (8823) and cv. Agria was inoculated with 5 to 6 *Dickeya* strains. Four hundred seed tubers were inoculated by soaking, planted in the field (year 1) and then, blackleg symptoms were assessed 12 to 16 weeks after planting. After harvest, 100 tubers from each plot were stored at 4°C and planted the following year and blackleg symptomatic plants were counted (year 2). This two year trial was repeated twice with respectively 5 and 6 *Dickeya* strains and thrice with respectively 5, 7 and 19 potato cultivars. For each trial, we tested the correlation between the expression of blackleg symptoms in year 1 and year 2. The results revealed that the correlation is better for aggressiveness trials (R-squared of 0.51 and 0.38) than for cultivar susceptibility studies (R-squared of 0.07, 0.06 and 0.21). This work indicates that the results of trials using lenticelar inoculation should be interpreted with caution, since they are not taking into account the infections of the progeny tubers through the vascular system of the plant. The results of trials using lenticelar inoculation are giving a partial indication of the susceptibility of one potato cultivar to various pectinolytic bacteria strains, but can reach to wrong conclusions if the objective of the trial is to distinguish the susceptibility of different cultivars to those bacteria.

Spreading of soft rot bacteria with nematodes and winter flies

Minna Pirhonen, Nykyri J. & Pasanen M.

Department of Agricultural sciences, University of Helsinki, Finland.

We have investigated the role of free-living nematodes and winter crane flies multiplying in storage rooms in the spreading of soft rot bacteria. Soft-rot enterobacteria were able to withstand nematode grazing, colonize the gut of *Caenorhabditis elegans* and subsequently disperse to plant material while remaining virulent. Two nematode species were also isolated from a rotten potato sample, and one of these isolates (*Pristionchus* sp.) was shown to be able to disperse soft-rot enterobacteria to plant material (Nykyri et al. 2013). The winter crane flies were collected from storage rooms and identified as *Trichocera maculipennis*. This species is known of antropogenic associations - in localities of cold climate it chooses relatively warm shelters, as cellars and caves, and often migrates with humans. No soft rot bacteria were identified in the adult winter crane flies collected from potato storage rooms. However, when the flies were exposed to potato tuber tissue containing soft rot bacteria, they were able to transfer the bacteria onto surface sterilized potato tubers. Furthermore, when the exposed potato tubers were incubated in rot-promoting conditions, they rotted faster than the control tubers. In conclusion, nematodes may have a role in the short-distance spreading of soft rot bacteria in soil, whereas the winter crane flies may spread the bacteria in potato storage rooms to clean harvest if rotten tubers are present in the same storage. Nykyri et al. 2013 Plant Pathology 63:747-757

Characterizing genetic resources to control the potato pathogen complex species belonging to *Pectobacterium* and *Dickeya*

Hélias Valérie [1, 2, 4], Quêtu-Laurent Angélique [1, 2, 4], Pellé Roland [3, 4], Le Hingrat Yves [1, 4], Marhadour Sylvie [1, 3, 4], Andrivon Didier [2, 4], Kerlan Marie-Claire [3, 4]

[1] *FN3PT/RD3PT, French Federation of Seed Potato Growers- Research, Development, Promotion of Seed Potatoes, 43-45 rue de Naples, F-75008 Paris, France*
[2] *INRA, UMR1349 IGEPP, F-35653 Le Rheu Cedex, France*
[3] *INRA, UMR1349 IGEPP, Keraiber, F-29260 Ploudaniel, France*
[4] *UMT Innoplant, UMR1349 IGEPP, F-35653 Le Rheu Cedex*

Pectobacterium and *Dickeya* are the two bacterial genera involved in blackleg and soft rot symptoms in potato. They cause major damage and costly losses in Europe and beyond. The use of genetic resistance constitutes an interesting prospect to control these diseases, which currently relies exclusively on prophylactic measures.

Progenitors with resistance to *P. atrosepticum* have been identified by INRA in the late 1990's, made available to the French potato breeders and introduced in useful genetic material (cultivars or hybrids) [1] [2]. However, annual surveys and diversity studies conducted by FN3PT-RD3PTin France over the last decade have shown evolutions in the prevalence and distribution of the species/subspecies complex responsible for soft-rot and blackleg [3]. One part of the National Research Program on *Pectobacterium* and *Dickeya* (FN3PT-RD3PT-INRA) aim at evaluating the performance of the resistance sources identified some years ago against *P. atrosepticum* to control the other members of this pathogenic bacterial complex. A collection of 24 hybrids from different genetic backgrounds was tested against representative strains of the main taxa responsible for soft rot and blackleg. Ten of these clones showed very low soft rot severity in front of two to five species/subspecies, and 9 additional clones showed specific resistance to one of these species. Experiments to test the resistance level of clones against blackleg (stem infections) will be conducted. These promising results open new hopes for the sustainable control of the soft rot/backleg complex in potato.

[1] Andrivon et al., 2003. Amer J Potato Res 80: 125-134

[2] Pasco et al., 2006. Potato Res 49 : 91-98.

[3] Hélias et al., 2014, 19th Triennial Conference of the EAPR, Brussels : p.110

The metabolic phases of the soft-rot disease

Nicole Hugouvieux-Cotte-Pattat

CNRS UMR5240 Microbiologie Adaptation et Pathogénie, Université de Lyon, Université Claude Bernard Lyon 1, INSA de Lyon, F-69621Villeurbanne.

The soft-rot disease caused by *Dickeya* dadantii is a dynamic process usually divided in two main phases. Bacteria penetrate their host using wounds or natural openings. During the first hours, bacteria reside latently in the plant intercellular space without provoking any symptoms. This "asymptomatic phase" is followed by a short transition to the "symptomatic phase" corresponding to appearance of the soft-rot symptoms.

Metabolomics (^{13}C-NMR spectroscopy) was used to analyze the metabolic activity of the pathogen. After infection of chicory leaves by *D. dadantii* 3937, the asymptomatic and the symptomatic phases corresponded to 1-8 hours and 12-24 hours, respectively. A kinetic analysis, with 4 h intervals, was performed to follow the infectious phases. The modifications observed during infection gave a direct image of the bacterial metabolism. During the asymptomatic phase, bacteria began to assimilate some easily metabolizable plant soluble sugars. A controlled repression of aggressive factors allows the bacteria to multiply without being recognized by their host. The transition step corresponds to a strong metabolic change including induction of pectate lyase production. The symptomatic phase corresponds to the expansion of soft-rot due to the pectate lyase burst. Bacteria multiply to large population sizes by obtaining more nutrients through the degradation of plant cell wall polysaccharides.

Whole-genome comparison-based classification of the soft rot *enterobacteriaceae*

Leighton Pritchard[1], Sonia Humphris[1], Rachel Glover[2], John Elphinstone[2] and Ian Toth[1]

[1]James Hutton Institute, Invergowrie, Dundee DD2 5DA
[2]Fera Science Ltd., York YO41 1LZ

Effective detection and diagnosis of the soft rot *Enterobacteriaceae* (SRE) is dependent on the ability to classify pathogenic isolates accurately. Diagnostics and classification are made more difficult by the influence of horizontal gene transfer on phenotype, and historically complex and sometimes inaccurate nomenclatural and taxonomic assignments that persist in strain collections and online sequence databases. We present novel whole-genome classifications of the SRE using average nucleotide identity (ANI), illustrating inconsistencies between the established taxonomies and evidence from completely sequenced isolates

Mechanism of antimicrobial activity of *Pseudomonas* sp. P482 against soft rot pathogens: a study aided by genome mining

Dorota M. Krzyżanowska, Adam Ossowicki, Magdalena Rajewska, Tomasz Maciąg & Sylwia Jafra

Laboratory of Biological Plant Protection, Intercollegiate Faculty of Biotechnology UG&MUG, Kładki 24, 80-822 Gdansk, Poland

Pectobacterium and *Dickeya* are plant-pathogenic bacteria causing soft rot disease of many vegetables and ornamental plants. Despite serious economic losses caused by these bacteria, the means to fight these pathogens are limited. *Pseudomonas* sp. P482, a Gram-negative bacterium originating from the rhizosphere of tomato, is able to inhibit the growth of various species of *Pectobacterium* and *Dickeya* in a plate assay and to attenuate plant tissue maceration caused by the selected strains of these pathogens.

To reveal the mechanisms involved in the antagonism between *Pseudomonas* sp. P482 and the soft rot pathogens, a transposon mutagenesis and a genome mining approach in combination with the site-specific mutagenesis were applied. The genome sequence of *Pseudomonas* sp. P482 was obtained, automatically annotated and subjected to data mining in search for secondary metabolite biosynthetic clusters. The search was performed both manually, with the blastp tool, and automatically, with the antiSMASH 2.0 software. Five genes were selected based on the *in silico* study, each representing one biosynthetic cluster predicted by the antiSMASH 2.0 as a result of a default search against its internal database. Knock out of these genes showed that they are not responsible for the antimicrobial activity of P482 against the soft rot bacteria. A more unrestricted antiSMASH 2.0 search based on Pfam domain probabilities revealed 18 more clusters designated as "hypothetical". A transposon mutant of P482 not inhibiting the growth of Dickeya spp. in vitro, carries an insertion in one of these "hypothetical" clusters. Thus, genome mining for secondary metabolite clusters is a powerful approach although when novel mechanism are at stake, it should be used in combination with other methods

Implementation of research results on *Erwinia* into the seed production in The Netherlands.

Kees Kristelijn

HZPC, Edisonweg 5, 8501 XG Joure

In the Netherlands, after the *Erwinia* problems in the early 2000's, a survey was held under 300 seed potato growers. After a scan of the problems, and the answers of the farmers in the survey, a project with all major stakeholders was started in 2005. During the periods 2005 – 2009 ("Bacterievrije pootgoedteelt") and 2009 – 2013 ("Deltaplan Erwinia") research was done on almost all aspects of growing seed potatoes. All this research lead to a list of do's and don'ts which had to be implemented within the process of growing seed potatoes. A group of 8 companies , with more than 90% of the total acreage of seed potato production in the Netherlands, started a new project called Deltaplan Erwinia 2.0. HZPC started to work with the results of the first project in 2009. Adjustments in advice's to growers, and testing of basic seed material, have lead to less problems in degrading and rejection of seed lots in the last years in the Netherlands. This, despite the growing concern about the development of P.c. subsp. *Brasiliense*. In the years after 2009 the development of Erwinia in the different HZPC seed lots was monitored. In my presentation I will highlight some results of the first year of Deltaplan 2.0 and go in to more detail in the way HZPC acts on the implementation of research results on Erwinia into the seed potato production in the different seed production area's in Europe.

Characterization of pectinolytic bacteria isolated in potato in Poland and Norway in 2013

Renata Lebecka[1], A. Grupa[1], M. Wiken Dees[2], J. Perminow[2], R. Nærstad[2], M. B. Brurberg[2], A. Motyka[3], S. Żołędowska[3], W. Śledź[3], M. Potrykus[3], M. Golanowska[3], R.Czajkowski[3], E. Łojkowska[3]

[1] *Plant Breeding and Acclimatization Institute – National Research Institute, Mlochow Division, Platanowa 19, 05-831 Mlochow, Poland*
[2] *Norwegian Institute of Bioeconomy Research, Høgskoleveien 7, NO-1431, Ås, Norway*
[3] *Intercollegiate Faculty of Biotechnology UG&MUG, Kładki 24, 80-822, Gdańsk, Poland*

Bacteria belonging to *Pectobacterium* and *Dickeya* spp. cause soft rot diseases of a great variety of crops and ornamentals worldwide. In the last 5 years potato losses caused by soft rot have increased significantly and it appears that warmer climate escalate the disease development. We investigated the population structure of pectinolytic bacteria (*Dickeya/Pectobacterium* species) in Poland and Norway. The survey was conducted in 2013, and a representative selection of species and isolates from both countries, were examined in detail. From Poland, 17 Pectobacterium wasabiae (Pw), 14 *P. atrosepticum* (Pba), 9 *P. carotovorum* subsp. *carotovorum* (Pcc), and 2 - *Dickeya solani* (Ds) were selected. From Norway, 21 Pcc and 18 Pa isolates were selected. Single isolates of Pw and Ds were found. The isolates were characterized for several phenotypic traits: the aggressiveness on potato tubers, production of cellulases, pectinases, proteases, siderophores, motility, and growth in the presence of high salt concentration (5% NaCl). A maceration test on tubers of the susceptible potato cultivar Irys, at 26oC, showed high variability in aggressiveness within each bacterial species. The isolates from Norway were in average more aggressive than the Polish ones. The Pcc isolates were significantly more aggressive than the Pba isolates, and these were more aggressive than the Pw isolates. Polish strains of Pba, Pcc, Pwa and Ds did not differ significantly from Norwegian strains in other analyzed phenotypic traits. Individual isolates exhibited differences especially in siderophore, protease production and motility. Only three of the analyzed Pba strains produced proteases. The prediction of bacterial species based on discriminant analysis of preliminary data of all phenotypic traits ranged from 0.667 (for Pwa) to 1.0 (for Ds). The work is in progress within Polish-Norwegian POTPAT project.

Mass spectrometry in microbiological research.

Katarzyna Macur, Paulina Czaplewska

Laboratory of Mass Spectrometry, Core Facility Laboratories, Intercollegiate Faculty of Biotechnology UG-MUG, ul. Kładki 24, 80-822 Gdańsk, Poland

Mass spectrometry (MS) is a powerful tool to investigate microbial world. New developments in MS instrumentation and analytical workflows have been contributing to wider application of this technique in different areas of microbiology, such as taxonomic identification or microbial interactions [1]. The presentation will focus on different applications of mass spectrometry equipment of the Laboratory of Mass Spectrometry, supported by the FP7 MOBI4Health project, in microbial protein research performed at the at the Intercollegiate Faculty of Biotechnology UG-MUG. The examples will include application top-down and bottom-up proteomic approaches for identification of intact proteins and protein digests, respectively. The SILAC-based protein identification and quantification of differently treated *Candida albicans* cells will also be described. Apart from presently used at the IFB UG-MUG MS workflows, new applications available on our instrumentation of particular interest for microbiological studies will be shown.

[1] T. Luzzatto-Knaan, A.V. Melnik, P.C. Dorrestein: Mass spectrometry tools and workflows for revealing microbial chemistry. *Analyst* 2015, 140:4949.

Biodiversity of *Dickeya* spp. isolated from potato plants and water sources in temperate climate

Marta Potrykus[1], M. Golanowska[1], W. Sledz[1], S. Zoledowska[1], A. Motyka[1], A. Kolodziejska[2], J. Butrymowicz[2] & E. Lojkowska[1]

[1] Laboratory of Plant Protection and Biotechnology, Intercollegiate Faculty of Biotechnology of University of Gdansk and Medical University of Gdansk, Kladki 24, 80-822 Gdansk, Poland.
[2]The Central Laboratory of the State Plant Health and Seed Inspection Service, Zwirki i Wigury 73 87-100 Torun, Poland

Bacteria from the genera *Dickeya* (formerly *Erwinia chrysanthemi*) and *Pectobacterium* (formerly *Erwinia carotovora*) are the agents of blackleg and soft rot disease on many important crops. In 2005, *D. solani* was isolated for the first time in Poland from a symptomatic potato plant. This motivated us to study the presence and diversity of *Dickeya* spp. in potato fields and water sources (including surface waters near potato fields, and water from potato processing facilities and sewage plants) in temperate regions of Poland (Central Europe). Only *D. dianthicola* and *D. solani* were isolated from symptomatic potatoes, and only *D. zeae* and *D. chrysanthemi* were isolated from water sources. The *Dickeya* spp. isolated from potato formed a relatively homogenous group, while those from water sources were quite diverse. To our knowledge, this is the first comprehensive characterization of *Dickeya* spp. isolated from regions with a temperate climate in Central Europe.

Survey of grower practice and identification of contributory factors which may be causing an increase in blackleg occurrence in Scotland

Gerry Saddler[1], Triona Davey[1] & Claire Hodge[2]

[1] SASA, Edinburgh EH129FJ, Scotland, UK
[2] AHDB Potatoes (Edinburgh), Scottish Office Rural Centre West Mains, Newbridge Midlothian, EH28 8NZ, Scotland, UK

Blackleg remains the biggest cause of down-grading and rejection in the Scottish Seed Potato Classification Scheme. Currently, control is reliant on initiating production from disease free planting material, industry good practice and the implementation of the Classification Scheme itself. In Scotland, healthy planting material arises from disease-tested micro-plants produced by SASA, which are multiplied at 8 mini-tuber production facilities, under controlled conditions, across the country. Field grown potatoes are initially produced as pre-basic (PB) seed by 28 registered growers, where a zero-tolerance for blackleg (and other faults) is applied. Crop inspections during every year of field multiplication serve to limit/remove heavily diseased crops from the production chain.

Up until 2007 this approach was judged to be effective however more recently blackleg incidence has risen steadily, peaking in 2011 to levels not seen for more than 20 years. In addition, post -harvest tuber testing of PB stocks has shown that some crops can become infected with *Pectobacterium atrospeticum* (in the absence of symptoms) as early as FG1. It is also clear that by looking at the national picture from field inspections returns of basic crops, the incidence of blackleg generally increases with each subsequent field generation.

The purpose of this study was therefore to investigate what influence, if any, social changes/industry practices may have on blackleg incidence with a view to identifying contributing factors, should they exist. The survey initially focused on Scotland's 28 PB growers (alongside 1 PB grower each from England and Northern Ireland) and was based on one-to-one interviews in which a series of questions around social, financial and economic change were addressed. Results from this part of the study highlight possible contributory factors which may in part be contributing to the rise in blackleg incidence. The next stage in the process will be described from which, it is hoped, a refined approach to blackleg control will be derived.

Dickeya solani and *Pectobacterium* spp. in Israel

Leah Tsror

Department of Plant Pathology and weed Research, Agricultural Research Organization (ARO), Gilat Research Centre, MP Negev 85280, Israel.

Potato is being cultivated in two major seasons in Israel; seed lots are imported from Europe for the spring each year whereas for the fall-winter season the growers use their own seeds (produced in spring under inspection of PPIS). Spring begins with low temperatures followed by increase, while the fall starts with high temp which decrease later. Pectinolytic bacteria cause economic losses with symptoms including pre-emergence tuber rot, blackleg and wilt. Although the pectinolytic bacteria are transmitted mostly by latent infection of seed tubers, disease symptoms vary with climate. *Dickeya solani* (Ds), *Pectobacterium carotovorum* (Pc), *P. c. brasiliense* (Pcb) and P. wassabiae (Pw) can grow at low and high temperatures with higher maximal growth temperature compared with *D. dianthicola* or *P. atrosepticum* (Pa). Development and expression of the disease in field may be particularly favored under warm-climate conditions.

The protocol for detecting *Dickeya*-latent infection in seed lots by enrichment RT-PCR was used to detect also other *Pectobacterium* spp. In 2015, 77 commercial imported seed lots were checked for latent *D. solani* and *Pectobacterium* spp. infections. Field surveys indicated that 69% of seed lots that were Ds-negative had no disease symptoms in fields (-L/-F), 19% were Ds-positive lots with field symptoms (+L/+F), 9% of seed lots were Ds-negative but had symptoms (false negative; -L/+F) and 3% were false positive (+L/-F). In a field trial conducted in 2015, seed tubers of randomly selected imported seed lots (10 lots of cv. Sifra, 6 Mozart, one of each cvs. Winston, Marabel, Rodeo, Canberra) were planted at Gilat (4 reps X 50 tubers). Wilt and blackleg symptoms were weekly monitored and the presence of pectinolytic bacteria was checked in seed tubers, plants and progeny tubers. In the selected seed lots, Ds and Pcb were detected in 75% and 55%, respectively. At the end of the experiment all sampled plants were contaminated with Ds, and 45% had Pcb. In progeny tubers, 75% were infected with Ds, and 70% with Pcb. Ware potatoes were recently exported to Jordan but only after lab test for Ds. Out of 39 tested lots 11 were Ds-positive (28%) and therefore rejected (among them: Sifra - 20 lots with 7 rejected; Winston 8 lots, 2 rejected; Panamera 2 lots, one rejected). The presence of Ds and Pcb in imported seed material directly affect yields in the spring crop in Israel, as well as on export of ware potato produced in this season in addition to the fall crop which might be also affected. Pathogenicity of Pcb isolates using the potato tuber maceration test was evaluated and further characterization will be carried out.

Comparison of *Dickeyas* expression profiles in macerated tubers and *Dickeya* solani diversity

Jacques Pédron[1], Yannick Raoul des Essart[2,3], Slimane Khayi[2,4], Valérie Hélias[3], Denis Faure[2] and Frédérique Van Gijsegem[1]

[1] *iEES Paris (Institute of Ecology and Environmental Sciences), INRA-UPMC Université Paris 06, UMR 1392, 7 quai Saint-Bernard F-75005, Paris, France*
[2] *Institut for Integrative Biology of the Cell (I2BC), CNRS-CEA-Université Paris-Sud, Saclay Plant Sciences, UMR9198, Avenue de la Terrasse, 91198 Gif-sur-Yvette cedex, France*
[3] *Research, Development, Promotion of Seed Potatoes - French Federation of Seed Potato Growers (RD3PT-FN3PT), 43-45 Rue de Naples, 75008, Paris, France*
[4] *Université Moulay Ismaïl, Faculté des Sciences, Département de Biologie, Meknès, Maroc*

Comparison of the complete genome sequence of one *D. solani* and one *D. dianthicola* strains, both isolated from diseased potato in France, revealed a high synteny between these genomes. Both genomes only harbour a few hundred genes present in only one of the two species. Interestingly, these species-specific genes are often clustered in genomic regions, frequently associated with mobile elements in *D. dianthicola*. Most of these genomic regions regroup genes that are predicted to be involved in metabolism/transport as well as regulatory genes. Comparison of gene expression profiles after tuber infection reveals that the expression of several of these species-specific genes is modulated in planta as compared to in vitro bacterial growth pointing to an involvement in plant-bacteria interactions. A different modulation of several genes that are common to both species was also observed in planta. This indicates specificities in gene regulation between both species and highlights the importance of expression analysis to unravel the complete diversity of related strains/species in their interaction with their hosts.

The *D. solani* diversity was analysed by comparing the complete genome sequence of *D. solani* strain 3337 with 19 other *D. solani* genomes of strains isolated in different years, from various geographical locations and from different hosts. This population genomic analysis highlighted an unexpected variability among *D. solani* isolates. Indeed, in addition to scattered SNP/InDel variations, replacing and additive horizontal gene transfers (HGT) were observed by inter-species introgression of *D. dianthicola* genomic regions and plasmid acquisition. Furthermore, this analysis led to the characterization of two distinct sub-groups within the *D. solani* species differing to each other by variations (mainly SNP/InDels) in about one half of the genes. Replacing HGTs were also observed between the two *D. solani* sub-groups.

Virulence of *Pectobacterium* and *Dickeya* species in potato under climate conditions in Western Europe

Jan van der Wolf[1], E.G. de Haan[2], P. Kastelein[1], M. Krijger[1], B.H. de Haas[1], H. Velvis[1], O. Mendes[1] & P.S. van der Zouwen[1]

[1] *Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands.*

[2] *Dutch General Inspection Service for Agricultural seed and seed potatoes (NAK), P.O.Box 1115, Randweg 14, 8300 BC Emmeloord, The Netherlands*

Dickeya and *Pectobacterium* strains, isolated from potato or from surface water in the Netherlands were tested in field experiments for their virulence in potato, Seed potatoes were vacuum-infiltrated and planted in 2013 and 2014 in randomized blocks in a clay soil in the Netherlands. Blackleg and slow wilt symptoms were recorded during the growing season. In both seasons, inoculation with strains of *Pectobacterium atrosepticum* and *P. carotovorum* subsp. *brasiliense* resulted in the highest disease incidences of between 75 and 95 percent of the plants that emerged. Inoculation with strains of *D. solani* and *P. wasabiae* resulted in disease incidences between 5 and 25 percent. Hardly any or no disease was observed in the treatments with four strains of *P. c* subsp. *carotovorum* strains, a strain of *D. dianthicola* and the water control. Co-inoculations of seed potatoes with a strain of *P. c.* subsp. *brasiliense* and *D. solani* gave a similar disease incidence as inoculation with *P. c.* subsp. *brasiliense* only. However, co-inoculation of *P. carotovorum* subsp. *brasiliense* with *P. wasabiae* decreased the disease incidence compared to inoculation with *P. c.* subsp. *brasiliense.* The presence of pathogens in progeny tubers of plants was confirmed with enrichment-TaqMan assays and for some treatments with dilution plating followed by a colony-TaqMan assay. For this, specific TaqMan assays were developed and evaluated for detection of *P. c.* subsp. *brasiliense* and *P. wasabiae* whereas a TaqMan for detection of *P. atrosepticum* previously developed previously at FERA (UK) was evaluated.

Plant phenolic acids affect the virulence of *Pectobacterium* spp. via quorum-sensing regulation

Janak Raj Joshi[1, 2], Alexander Lipsky[2], Shaked Yariv[2] and Iris Yedidia[2]*

[1] *Department of Plant Pathology and Microbiology and the Otto Warburg Minerva Center for Agricultural Biotechnology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel*
[2] *Department of Plant Sciences, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel*
[*] *Correspondence. E-mail: irisy@volcani.agri.gov.il*

The virulence strategy of *Pectobacteria* is based on the secretion of exoenzymes that degrade the cell wall of their hosts, providing nutrients to the bacteria on the one hand, but conversely exposing the bacteria to plant defense compounds on the other. To better understand the effects of these plant-derived antimicrobial molecules, we have screened phenolic acids and polyphenols, for their ability to affect virulence in several *Pectobacteria*. The results revealed a significant effect of some phenolic compounds on virulence determinants such as motility, biofilm formation and extracellular enzyme activities. Moreover, short exposure to some phenolics prior to infection reduced disease severity by 20 to 100%, as shown in infection assays in three different hosts. The effect was not species specific and was proven effective in *P. carotovorum*, *P. carotovorum* subsp. *basiliense*, *P. atrosepticum* and *P. aroidearum*. To explore the mechanism underlying these processes, the effect of cinnamic acid (CA) and salicylic acid (SA) on virulence was further investigated in *P. aroidearum* and *P. carotovorum* subsp *brasiliense*. The results clearly indicated that both compounds interfered with the quorum-sensing (QS) machinery of the two species, consequently altering the expression of bacterial virulence factors. While in control treatments, expression of QS-related genes increased over time, exposure of bacteria to nonlethal concentrations of CA or SA inhibited the expression of QS genes, including *expI*, *expR*, PC1_1442 (*luxR* transcriptional regulator) and luxS (a component of the AI-2 system). Other virulence genes known to be regulated by the QS system, such as *pecS*, *pel*, *peh* and *yheO*, were also down-regulated relative to the control. In agreement with the low levels of expression of *expI* and *expR*, CA and SA also reduced the level of N-acyl-homoserine lactone (AHL) signal. The effects of CA and SA on AHL signaling was confirmed in compensation assays, in which exogenous application of N-(β-ketocaproyl)-L-homoserine lactone (eAHL) led to the recovery of the reduction in virulence caused by the two phenolic acids. The results support a mechanism by which plant phenolics interfere with *Pectobacterium virulence* via the QS machinery.

Bacterial diseases of potatoes in Chile: A re-emergent sanitary problem

Ivette Acuña, Sandoval, C. and S. Mancilla

Instituto de Investigaciones Agropecuarias, INIA Remehue. Casilla, 24-O, Osorno, Chile.

Potato crop in Chile is positioned in fourth place with an average area in the last 10 years of 51.929 ha, reaching a production and yield average of 1.130.405 tons and 21.9 t / ha (ODEPA, 2014). Considering fresh consumption, potato represents about 50 kg/inhabitant per year. In addition, this crop has a great economic and social importance being produced by nearly 60.000 farmers, 50% of them in southern Chile. Today, the main sanitary problems in potato are late blight, PVY and bacterial diseases. During the 90´s, *Pectobacterium atrosepticum* (*Erwinia carotovora* sp *atroseptica*) was described as the main causal agent of soft rot and black leg, with a disease incidence of 40% to 100% as tuber latent infection and producing 20% of yield losses. At that time, the problem was managed using in vitro multiplication in the potato seed production system. However, black leg and soft rot is increasing again in the last 5 years, producing 15% of yield losses mainly due to the use of new irrigation systems, favorable weather conditions and new varieties, among others. During the last potato seasons a new survey was performed to do a collection of plant and tuber isolates. Strains were characterized using specific PCR primer ECA1f + ECA2r (de Boer et al, 1995) and EXPCCF+EXPCCR (Kang et al, 2003) for *P. atrosepticum* and *P. carorotovorum* subsp *carotovorum*. PCR shows that 13.5% and 64.8% of the strains amplified with ECA1f + ECA2r or EXPCF+EXPCR primer, respectively. However, 10% of strains did not shows reaction with these primers. Then, these results indicate that new strains are associated with potato causing black leg and soft rot, however, new studies need to be performed to have conclusive results.

Characterization of Finnish Pectobacterium carotovorum isolates from potato stems showing blackleg symptoms

Miia Pasanen & Minna Pirhonen

Department of Agricultural sciences, University of Helsinki, Finland.

Pectinolytic enterobacteria isolated from potato stems and tubers in Finland were identified as *Pectobacterium atrosepticum*, *Pectobacterium wasabiae* and *Pectobacterium carotovorum* subsp *carotovorum* using phylogenetic analyses and biochemical tests (Pasanen et al. 2013). *P. c*. subsp. *carotovorum* strains clustered into two distinct groups in phylogenetic analyses. One of these groups mainly contained strains isolated from potato tubers and the other one mainly included isolates from potato stems. However, none of the *P. c.* subsp. *carotovorum* isolates differed statistically from the water-treated control in the amount of blackleg symptoms in a field assays with vacuum -inoculated seed tubers. Stem isolates were not able to elicit a hypersensitive response in tobacco leaves and they also produced barely a measurable levels of autoinducers in the stationary phase in vitro. Biolog phenotypic tests showed no differences between stem and tuber isolates. In DNA -DNA hybridization analysis stem isolate strain showed around 70% DNA-DNA relatedness with *P. c.* subsp. *carotovorum* type strain and in average nucleotide analysis they had less than 95% pairwise similarity value with any *P. carotovorum* species or subspecies, which is less than the cut off value (>95%) for species. Stem isolates clearly differ from *P. c.* subsp. *carotovorum* type strain and do not clearly resemble any other the *P. c. subspecies*, including *P. carotovorum* subsp. *brasiliense*. However, the stem isolates produced the expected amplicon with *P. c.* subsp. *brasiliensis* subspecies-specific primers, suggesting that these kind of strains can be mistakenly identified as *P. carotovorum* subsp. *brasiliense*.

Pasanen et al. (2013) Annals of Applied Biology 163(3):403-419.

Test methods and field correlations for Dickeya and Pectobacterium spp.

Eisse de Haan and Miriam Kooman

Dutch General Inspection Service for agricultural seeds and seed potatoes (NAK)

Results of the yearly *Pectobacterium* and *Dickeya* survey showed a the relative increase of *Pectobacterium carotovorum* subsp. *brasiliense* as causal agent of blackleg. Therefore, we performed field experiments to determine the aggressiveness of Pcb as compared to *Pectobacterium atrosepticum* and *Dickeya solani*. In addition, different test methods were compared and field correlations of these methods were determined. Results of these experiments will be presented.

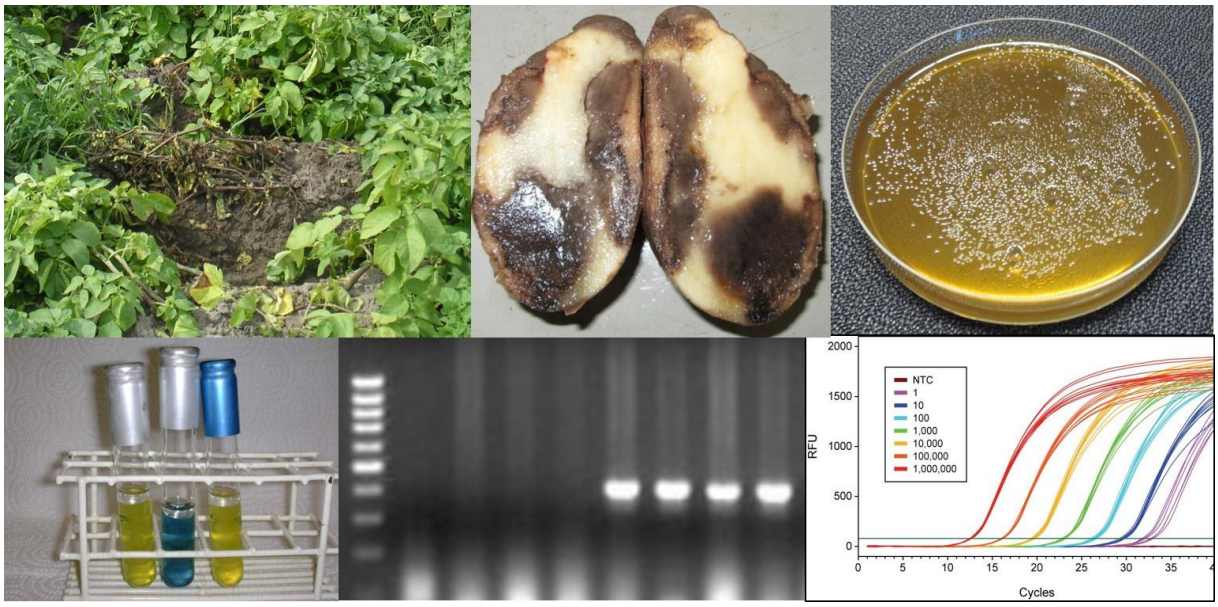
Euphresco
Network for phytosanitary research coordination and funding

Euphresco III

Assessment of *Dickeya* and *Pectobacterium* spp. on vegetables and ornamentals

10-12 November, 2016
University of Helsinki
Finland

Programme and abstracts



Programme

Date	Time	Activity	Speaker or Address
THURSDAY **November 10**	13:00	Arrival to Department of Agricultural Sciences Coffee and sandwich	Latokartanonkaari 5, 3rd floor, Viikki campus Room 336
	13.15	Welcome and practical information	Minna Pirhonen and Jan van der Wolf
		PLANT-PATHOGEN INTERACTIONS, ECOLOGY AND MANAGEMENT	
	13.30	Motility and chemotaxis: phenotypic analysis of *Dickeya solani*, comparison with Dickeya dadantii 3937	Nicole Hugouvieux-Cotte-Pattat
	13.50	Competitive fitness: An important survival strategy for Pcb1692 survival?	Lucy Moleleki
	14.20	Elucidating a mechanism by which plant derived small molecules affect quorum sensing related virulence targets of *Pectobacterium*	Iris Yedidia
	14.40-15.00	**Coffee break**	
	15.00	Transcriptome profiling of potato tubers as response to biological control bacterium *Serratia plymuthica*	Iman Hadizadeh
	15.20	Are waterways important reservoirs of phytopathogens for the genera *Dickeya* and *Pectobacterium* spp.?	Agata Motyka
	15.40	R&D into blackleg caused by *Pectobacterium atrosepticum*	Ian Toth
	16.00	Bacteriophages of Soft Rot Enterobacteriaceae – what we know so far?	Robert Czajkowski
	16.40	Characterization of the pathogens that cause blackleg of potato in Maine and their responses to chemical treatments and varieties	Jianjun Hao
	17.00	Core and accessory genomes in the *Dickeya* genus	Frédérique Van Gijsegem
	20.00	**Buffet dinner at Restaurant Kitzens**	Hotel Radisson Blu Plaza

FRIDAY November 11	**8.00**	**Leaving from the hotel**	
	8.17	Buss no 68 to Viikki leaving 8.17 Get off the bus by black gorilla	Railway square, platform 1
		DIAGNOSTICS AND SURVEYS	
	9.00	Improving diagnostics of soft rot Enterobacteriaceae	Jan van der Wolf
	9.20	Evaluation of real-time assays for the detection of *Pectobacterium* and *Dickeya*	Valérie Helias
	9.40	Classification of potato blackleg and soft rot disease in Canada	Sean Li
	10.00	Predicting Dickeya by post-harvest seed lot testing: A Real World Study	Gary Secor
	10.30-11.00	**Coffee break**	
	11.00	Pectobacterium and Dickeya situation in the potato crop in Chile	Ivette Acuña
	11.20	*Pectobacterium* and *Dickeya*: use of big and small data	Miriam Kooman
	11.40	Diagnostic microarray in parallel detection of bacterial pathogens of potato: hype or opportunity	Yeshitila Degefu
	12.00	Detection of *Pectobacterium* and *Dickeya* species in irrigation water in South Africa	Natalie Laughton
	12.20	One year survey of soft rot enterobacteria in non host environment	Marie-Anne Barny
	13.00-14.30	**Lunch break**	Lunch at the student and staff restaurant Ladonlukko
	14.30	Blackleg in Scotland: Recent findings	Greig Cahill
	14.50	*Dickey solani*, isolated occurrences in Norway?	Juliana Perminow
	15.10	Novel *Dickeya* species - again?	Špela Alič
	15.30	**Coffee, cake and discussions**	Jan van der Wolf Staff lunch room
	17.00	Finishing the meeting	
SATURDAY November 12	9.40-9.55	**Boat to Suomenlinna**	Market square by the water
	10.00-11.30	Guided tour in Suomenlinna	
	11.30-	Lunch	Suomenlinna
	13.20-13.35	Boat back to market square	Suomenlinna pier
	13.35-16.30	**Guided tour down town Helsinki**	At the market place

Abstracts

Motility and chemotaxis:
genomic and phenotypic analysis of *Dickeya solani*, comparison with *Dickeya dadantii* 3937

Nicole HUGOUVIEUX-COTTE-PATTAT

CNRS UMR5240 Microbiologie Adaptation et Pathogénie, Université de Lyon, Université
Claude Bernard Lyon 1, INSA de Lyon, F-69621Villeurbanne
Email: nicole.cotte-pattat@insa-lyon.fr

Motility and chemotaxis are crucial for the colonization of the host plant and a successful infection. Chemoreceptors (MCPs) are the sensory core of the system; they sense and transduce the signal to the flagellar motor. A bioinformatics analysis predicted 43 complete MCP genes into the *D. solani* genome. Each gene has an orthologue among the 47 MCP genes of D. dadantii and pairs of orthologues share the same genomic context. An experimental approach was also conducted to identify compounds able to induce chemotaxis in *D. solani* and dadantii. This phenotypic analysis showed a positive chemotactic response towards several sugars, amino acids or organic molecules, with few differences between strains or species.

Competitive fitness: An important survival strategy for *Pcb1692* survival?

MOLELEKI LN[1,2*] TANUI CK[1] AND SHYNTUM D[1]

[1]Department of Microbiology and Plant Pathology,
[2]Forestry and Agriculture Biotechnology Institute
University of Pretoria, Lunnon Rd, Pretoria
Email: lucy.moleleki@up.ac.za

Pectobacterium carotovorum subsp *brasiliense* (Pcb) is an important emerging soft rot and blackleg pathogen of potato plants globally, for which relatively little is known. This work uses the sequenced genome strain (Pcb1692) to investigate important virulence factors of this emerging pathogen. Towards this end, global genome-wide transcriptome profiling (RNA_Seq) of Pcb cells, during colonisation of potato tubers, was undertaken. From the data generated, it is evident that Pcb1692 utilises various strategies to kill or inhibit competing 'non-self' neighbouring bacteria cells residing within potato tubers. This presentation will explore these transcriptome data in more detail and discuss arising hypotheses.

Elucidating a mechanism by which plant derived small molecules affect quorum sensing related virulence targets of *Pectobacterium*

YEDIDIA I[1*] AND JOSHI RJ[1,2]

[1]The Institute of Plant Science, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel; [2]Department of Agro-ecology and Plant Health, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel
Email: irisy@volcani.agri.gov.il

The potential of plant derived phenolic molecules to control soft-rot caused by *Pectobacterium spp.* was investigated. To date, efforts to control the disease were mainly dedicated to improve detection methods and sanitation during storage and agricultural practice, with a limited degree of success. Here we tested the antimicrobial potential of different active phenolics on *Pectobacterium,* in search for a possible mode of action. Interestingly, biofilm formation and exoenzymes activity were significantly impaired, at compounds concentration that did not affect bacterial cell growth. These observations suggested a mechanism which specifically interferes with bacterial virulence. Since, these major virulence determinants (biofilm and exoenzymes) are controlled by quorum-sensing (QS), we focused on the effect of specific molecules on QS system in pectobacteria. The study revealed an inhibiting effect of the tested compounds on the expression level of central QS system and QS controlled genes, using quantitative real time-PCR. Two reporter strains (CV026 and pSB401) demonstrated a prominent reduction in the level of QS signal molecules N-acyl-homoserine lactone (AHL) accumulation, following exposure to the compounds. Moreover, infection capability was strongly impaired on three different hosts, potato, cabbage and calla-lily; but almost completely recovered upon external application of AHL (exogenous-AHL). To support potential interaction of the plant phenolic compounds with QS targets, drug discovery tools were used (SCHRODINGER®) to reconstruct a computational model of the QS central proteins in *Pectobacterium* ExpI/ExpR and predict the potential of specific compounds to bind to the active site of these targets.

Transcriptome profiling of potato tubers as response to biological control bacterium *Serratia plymuthica*

Iman Hadizadeh, Bahram Peivastegan and Minna Pirhonen

Department of Agricultural Sciences, PO BOX 27, 00014 University of Helsinki, Finland
Email iman.hadizadeh@helsinki.fi

One of the biggest quality problem in cultivation of potato and vegetables is caused by soft rot bacteria in the genera *Pectobacterium* and *Dickeya*. Biocontrol bacterium *Serratia plymuthica* A30 has a potential to control potato tuber soft rot caused by *Dickeya solani* at postharvest storage conditions. In this work, potato tubers were treated with A30 and potato transcriptome profiling was performed with RNA-seq to understand the functional response of potato tuber to this biological control bacterium. As a result of the biocontrol treatment, expression of genes involved in photosynthesis, response to stress, hormone signalling pathways and plant defence were induced in the tuber tissue.

Are waterways important reservoirs of phytopathogens for the genera *Dickeya* and *Pectobacterium* spp.?

MOTYKA A, SERBAKOWSKA K, BABINSKA W, ZOLEDOWSKA S, SLEDZ W, LOJKOWSKA E

[1]*Department of Biotechnology, Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, Gdansk, Poland*
Email: agata.motyka@biotech.ug.edu.pl

Pectinolytic bacteria from the genera *Dickeya* and *Pectobacterium* are causative agents of soft rot and blackleg on potato. Few transmission pathways of these pathogens were reported so far i.a. via the waterways. In this work we examined the survival of *Dickeya* and *Pectobacterium* spp. in Polish waters of different pollution status, both rivers and lakes. Additionally, information about the occurrence of pectinolytic bacteria in different waterways and different depths of the water reservoirs will be presented ed. Phenotypic features and the ability to cause potato tissue maceration of the isolated strains was evaluated. The presented data can open debate about the possible contribution of water-derived isolates to economic losses in potato production sector.

R&D INTO BLACKLEG CAUSED BY *PECTOBACTERIUM ATROSEPTICUM*

I K Toth[1], G Cahill[2], J G Elphinstone[3], S Humphris[1], G S Saddler[2] and S J Wale[4]

[1]*James Hutton Institute, Invergowrie, Dundee DD2 5DA*
[2]*Science and Advice for Scottish Agriculture (SASA), Roddinglaw Road, Edinburgh, EH12 9FJ*
[3]*Food and Environment Research Agency (Fera), Sand Hutton, York, YO41 1LZ*
[4]*SRUC Aberdeen Campus, Craibstone Estate, Aberdeen AB21 9YA*
E-mail: ian.toth@hutton.ac.uk

Pectobacterium atrosepticum continues to be the major issue for potato production in Northern Britain, while other species and genera are of concern in mainland Europe. A three year project, funded by AHDB Potatoes and the Scottish Government, has recently been commissioned to identify how and when early field generations become infected by *P. atrosepticum*, together with the effectiveness of sulphuric acid in haulm destruction. The project has 5 main components:-

- Monitor commercial first generation crops through a 3 year multiplication cycle.
- Investigate the movement of *P. atrosepticum* from infected to healthy plants.
- Investigate the routes by which daughter tubers become infected once *P. atrosepticum* is present in or on a plant.
- Identify whether a change in the population of *P. atrosepticum* strains has occurred in recent years.
- Use of modelling to better understand the geographical distribution of infected seed crops.
- Compare the effectiveness of sulphuric acid with currently used haulm destruction programmes to determine their relative impact on spread of *P. atrosepticum* to daughter tubers.

This presentation will summarise the findings from the project.

Bacteriophages of Soft Rot Enterobacteriaceae – what we know so far?

Robert Czajkowski, Anna Smolarska, Zofia Ozymko

Department of Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Abrahama 58, 80-307 Gdansk, Poland
e-mail: robert.czajkowski@biotech.ug.edu.pl

Soft rot *Enterobacteriaceae* (SRE: *Pectobacterium* spp. and *Dickeya* spp., formerly pectinolytic *Erwinia* spp.) are ubiquitous necrotrophic bacterial pathogens that infect a large number of different plant species worldwide, including economically important crops. Despite the fact that SRE bacteria have been studied for more than 50 years, little is known about their corresponding predators: lytic and lysogenic (temperate) bacteriophages. Lytic bacteriophages are able to shape the structure of host population and therefore may influence bacterial virulence, spread, persistence in environment and evolution. Up till now, ca. 6000 different individual bacteriophage isolates have been described and visualized by transmission electron microscopy (TEM), of which about 2000 (ca. 30%) target members of the *Enterobacteriaceae* family. In contrast, the number of characterized bacteriophages infecting the soft rot *Enterobacteriaceae* (SRE) bacteria (*Pectobacterium* spp. and *Dickeya* spp.) is much fewer and less than twenty. Likewise, the first (complete and/or draft) genomes of SRE 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. with the major focus on isolation of (novel) lytic bacteriophages from environmental samples and their morphological, proteomic, phenotypic and phylogenetic characterization. As well, the use of next generation sequencing and bioinformatic technologies in analyses of bacteriophage genomes and comparative phage genomics will be discussed.

Characterization of the pathogens that cause blackleg of potato in Maine and their responses to chemical treatments and varieties

Hao JJ[1] Marangoni N[1] Jiang H[1] Song Y[1] Ge T[1] and Johnson SB[2]*

[1]School of Food and Agriculture,
[2]Cooperative Extension
University of Maine, Orono, ME 04469, USA
**Email jianjun.hao1@maine.edu*

Blackleg of potato is a newly emerging disease in the Eastern states of the US. The pathogen population consisted of *Dickeya dianthicola, Pectobacterium wasabiae,* and *P. caratovorum* being the major species. *D. dianthicola* was the predominant species and could be the reason of the outbreak since 2015. The potato variety 'Sebago' was the most tolerant to *P. carotovorum*; AF5312-1 was the most tolerant to *P. wasabiae*; 'Yukon Gold', AF 4172-2, and 'Shepody' were the most tolerant to *Dickeya* spp. In chemical treatments, oregano essential oil and isothiocyanate inhibited both *Dickeya* spp. and *Pectobacterium* spp.

Core and accessory genomes in the Dickeya genus

PÉDRON Jacques and VAN GIJSEGEM Frédérique
Institute of Ecology and Environmental Sciences – Paris
INRA-UPMC-CNRS-IRD-Université Paris Diderot-UPEC
Paris, France Email: vangijse@agroparistech.fr

Multiple sequencing of genomes belonging to a bacterial genus or species allows one to analyse and compare the gene complements of genus or species, their pan-genomes. Such a study reveals species diversity and gene families that may be of special interest, e.g because of their role in bacterial survival/virulence or their ability to discriminate strains. We began such an analysis for the *Dickeya* genus by comparing 23 genomes available in databases using a SILIX-based clustering of the ORFs sharing at least 80% identity using Blastp scores. This allowed us to define a Dickeya genus core genome including the genes shared by all the analysed bacterial strains as well as core genes shared by different phylogenetic clades in the *Dickeya* phylogenetic tree. We also identified the genes that are specific to the different *Dickeya* species. These data will be discussed in terms of species specificity and link to virulence potential.

Improving diagnostics of soft rot *Enterobacteriaceae*

Jan van der Wolf, Marjon Krijger, Pieter Kastelein, Lia de Haas, Patricia van der Zouwen, Theo van der Lee, Peter Bonants, Elio Schijlen, Jan Bergervoet

[1] Wageningen Plant Research
P.O. Box 16, 6700 AA Wageningen, the Netherlands
Email Jan.vanderWolf@wur.nl

Testing of plant propagation material for soft rot *Enterobacteriaceae* (SRE), but also studies on ecology and disease management, require the availability of reliable diagnostic methods. Methods for characterization and testing of plant propagation material currently in use or recently developed will be shortly evaluated. These include methods for 1. Isolation, 2. characterization of bacteria using multilocus sequence analysis, 3. testing for virulence, 4. Enrichment of SRE, 5. detection with TaqMan and LAMP assays, 6. multiplex detection with the (Luminex) xTaq technology, and 7. NGS-analysis in complex substrates.

Evaluation of real-time assays for the detection of *Pectobacterium* and *Dickeya*

HELIAS V [1, 2,] QUETU-LAURENT A [1, 2,] CHAWKI K [1, 2,] AND ANDRIVON D [2]

[1] FN3PT/RD3PT, French Federation of Seed Potato Growers /Research, Development,
Promotion of Seed Potatoes, 43-45 rue de Naples, F-75008 Paris, France
[2] INRA, UMR1349 IGEPP, F-35653 Le Rheu Cedex, France
Email: valerie.helias@fnpppt.fr

The French Federation of Seed Potato Growers leads, in collaboration with INRA, a research program on *Pectobacterium* and *Dickeya* including assessment and development of methodologies for the detection of bacterial species involved in potato crop damages. The presented work aimed to evaluate the specificity and the sensitivity of several real-time PCRs protocols. The study focused on 7 real-time of the 11 assays initially selected for the Euphresco ring test for the detection of *P. atrosepticum*, *Dickeya* spp., *D. solani*, *D. dianthicola*, *P. brasiliense* and *P. parmentieri*, including Taqman and Sybergreen technology. The test performance study evidenced differences in specificity and sensitivity. Comparisons will be enlarged to other real-time assays in case of the availability of alternative tests for the detection of the same target organisms.

Classification of potato blackleg and soft rot disease in Canada

LI X, NIE J, ARSENAULT, H, XU H, AND De BOER SH.

Canadian Food Inspection Agency, Charlottetown Laboratory
Email: sean.li@inspection.gc.ca

Dickeya spp. and *Pectobacterium* spp. have caused substantial losses globally due to the emergence of new species/subspecies, and the wide spread dispersion of these pathogens which are more virulent and pathogenic in a wide variety of hosts. Particularly in potatoes, *Dickeya* spp and *Pectobacterium* spp can cause blackleg and blackleg-like diseases which have become increasingly problematic for seed potato production in many potato growing regions. In this report, we summarized the identification, characterization and classification of potato isolates of these two genera obtained during the last few years. In North America, *P. atrosepticum, P. kelmani (a potato isolate of P. wasabiae)***,** and *P. carotovorum* subsp. *brasiliensis* were routinely detected during the seed potato indexing, whereas *Dickeya* spp are relatively rare in seed potato lots. *D. solani* has not been detected in potatoes in Canada. However, *Dickeya solani* infecting hyacinth bulbs imported from overseas may be a potential threat to the local potato industry.

Predicting Dickeya by post-harvest seed lot testing: A Real World Study

GARY SECOR[1], JOHN NORDGAARD[2] and VIVIANA RIVERA[1].

[1]Department of Plant Pathology, North Dakota State University, Fargo, North Dakota, USA 58102, [2]Black Gold Farms, Grand Forks, North Dakota, USA 58201

Stand losses due to *Dickeya dianthicola* is new in the USA. Most infection of seed lots is latent and cannot be detected visually, and seed lots that appear free of Dickeya may exhibit serious stand losses after planting. Post-harvest PCR testing can detect latent seed infection, but there is little data connecting latent Dickeya seed infection with Dickeya in the field. We used PCR to test tuber samples of seed lots destined for commercial planting and monitored stand losses due to Dickeya. There appears to be a strong association between latent Dickeya infection and stand losses in commercial fields. In the USA, it appears that post-harvest seed lot testing can be used to predict stand losses due to Dickeya.

Pectobacterium and *Dickeya* situation in the potato crop in Chile

ACUÑA, I.[1]; GUTIÉRREZ, M.[2]; SANDOVAL, C.[1] and S. MANCILLA[1]

Instituto de Investigaciones Agropecuarias, INIA Chile.
Laboratorio Regional Osorno, Servicio Agrícola y Ganadero SAG Chile
E-mail: iacuna@inia.cl

Bacterial diseases in the potato crop have been increasing in the last years, due to various factors, among those the quality of potato seed, the use of irrigation systems and the climate change. These factors have led to an increase in the incidence and severity of black and soft rot caused by *P. atrosepticum* and *P. carorotovorum* subsp *carotovorum* with losses of up to 30% on susceptible cultivars and rejection of seed lots up to 24%. Added to this, *Dickeya* has been described in table stock potato production in the central part of the country. Due to this situation, it is necessary to develop epidemiology studies, seed diagnostic techniques and integrated management strategies to solve this sanitary problem.

Pectobacterium and *Dickeya*: use of big and small data

Miriam Kooman and Robert Vreeburg.

Dutch General Inspection Service for agricultural seeds and seed potatoes (NAK)

Since 2012, large scale-post harvest tests have been performed on *Dickeya* and *Pectobacterium* in Dutch seed potatoes. Both the field inspection results and data from the post-harvest tests are now available in a data warehouse and can be combined and analysed to find predictive correlations. Initial results from these analyses will be presented, like year-to-year correlations, lab-to-field correlations and (anonymised) variety data. The data can be combined with data from external sources to perform (big) data analyses.

Diagnostic Microarray in Parallel Detection of Bacterial Pathogens of Potato: hype or opportunity

YESHITILA DEGEFU

Natural Resources Institute Finland,
Green Technology, Biotechnology and Genetic Resources
Paavo Havaksen tie 3, FI-90014 University of Oulu, Finland.
yeshitila.degefu@luke.fi

Mixed infections with different pathogens are common in plants, which challenges pathogen detections by increasing analysis cost and time. Therefore, methods allowing parallel detection of different pathogens are needed. Potato tubers are infected with many bacterial pathogens whose detection is necessary in seed certification and quality control of ware potato stocks. In this study, a diagnostic microarray previously tested for specificity of probes for detection of eight known major potato bacterial pathogens was evaluated for routine and end user applications. Results of investigations involving tuber samples and samples emulating actual sample (tuber) are presented. While diagnostic microarray should not be considered unfit, it does not quite live up to the hype either. Details will be discussed.

Detection of *Pectobacterium* and *Dickeya* species in irrigation water in South Africa

LAUGHTON NH, CHIDAMBA L AND VAN DER WAALS JE

Plant Pathology Programme, Department of Plant and Soil Sciences,
Private Bag X20, Hatfield, Pretoria, 0028, South Africa
Email: nat.laughton@gmail.com, Jacquie.vdwaals@up.ac.za

Soft rotting Enterobacteriaceae (SRE) such as *Pectobacterium* and *Dickeya* spp. are responsible for causing black leg and soft rot diseases of potatoes. These diseases are of major concern to the potato industry throughout the world. Detection and identification of *Pectobacterium* and *Dickeya* species in water sources used for irrigation is extremely important for management of the diseases. In South Africa no studies have been done to identify the presence or prevalence of either genus in water systems. Five potato growing regions in South Africa were sampled. Water samples from dams, boreholes, rivers and overhead irrigation systems were surveyed for *Pectobacterium* and *Dickeya* species. Identification was done by REP-PCR and Multiplex PCRs, using species specific primers. Initial tests revealed that SRE are detectable in water systems used for irrigation in most regions sampled. Notably SRE were detectable in all overhead irrigation systems sampled. This is an important finding for the South African agricultural industry.

One year survey of soft rot enterobacteria in non host environment

BARNY MA[1], VAN GIJSEGEM F[1] and BERGE O[2]

[1] Institute of Ecology and Environmental Science-Paris
University Pierre and Marie Curie - 4 place Jussieu - 75252 PARIS cedex 05,
[2]INRA, Plant Pathology Research Unit, 84143 Montfavet, France
Email: barny@agroparistech.fr; barny@upmc.fr

Soft rot enterobacteria (SRE: *Pectobacterium* spp. and *Dickeya* spp.) infect a large number of plant species worldwide, including economically important plants. While the diversity of SRE observed on plant is fairly well described, the presence and diversity of SRE outside the plant context is not known in detail. In this study we sampled SRE outside the plant context. In october 2015, february, may and august 2016 we sampled on 21 sites along the river Durance in the south of France. Nearly 300 strains were isolated and we will present the results of this year survey.

Blackleg in Scotland: Recent findings

GREIG CAHILL AND GERRY SADDLER

Science and Advice for Scottish Agriculture (SASA)
Edinburgh, Scotland (UK)
Email: greig.cahill@sasa.gsi.gov.uk & gerry.saddler@sasa.gsi.gov.uk

Blackleg continues to be the principal cause of downgradings and rejections of seed crops from the Scottish seed potato classification scheme (SPCS). In Scotland, unlike other parts of Europe, *Pectobacterium atrosepticum* remains the predominant pathogen found. Multi-locus sequence analysis (MLSA) confirms that although *P. atrosepticum* is a diverse species there is little evidence to suggest that new pathogenic groups have emerged to explain the recent increase in disease levels. Results from MLSA may also indicate that environmental sources are just as important and seed-borne infection. These and other results are helping to shape the SPCS and during the 2016 growing season seed classification scheme rules were tightened, introducing a third inspection for ‘at risk’ crops. Initial results from this trial will be discussed in addition to outlining future plans.

Dickey solani, isolated occurrences in Norway?

J.I.S. Perminow, M.B. Brurberg
NIBIO Biotechnology and Plant Health
Norway
Email: juliana.perminow@nibio.no

Potato blackleg and soft rot are bacterial diseases of potato. Symptoms include dark discoloration of the stem base and aerial stem rot. Damaged vascular tissue leads to wilting of the affected plants. Soft rotting of tubers also occurs. There are several bacteria that can cause the described symptoms, *Pectobacterium* sp. and *Dickeya* sp. In 2005, a new aggressive *Dickeya solani* was detected in the Netherlands, and in the following years in the majority of potato producing countries in Europe.
In Norway, an isolated detection of *Dickeya solani* took place in 2012 in the course of an official survey. The finding was made in potato plants grown from imported seed potatoes under quarantine conditions, after which plants were destructed. In 2015 *Dickeya solani* was detected in three Norwegian field trial locations.

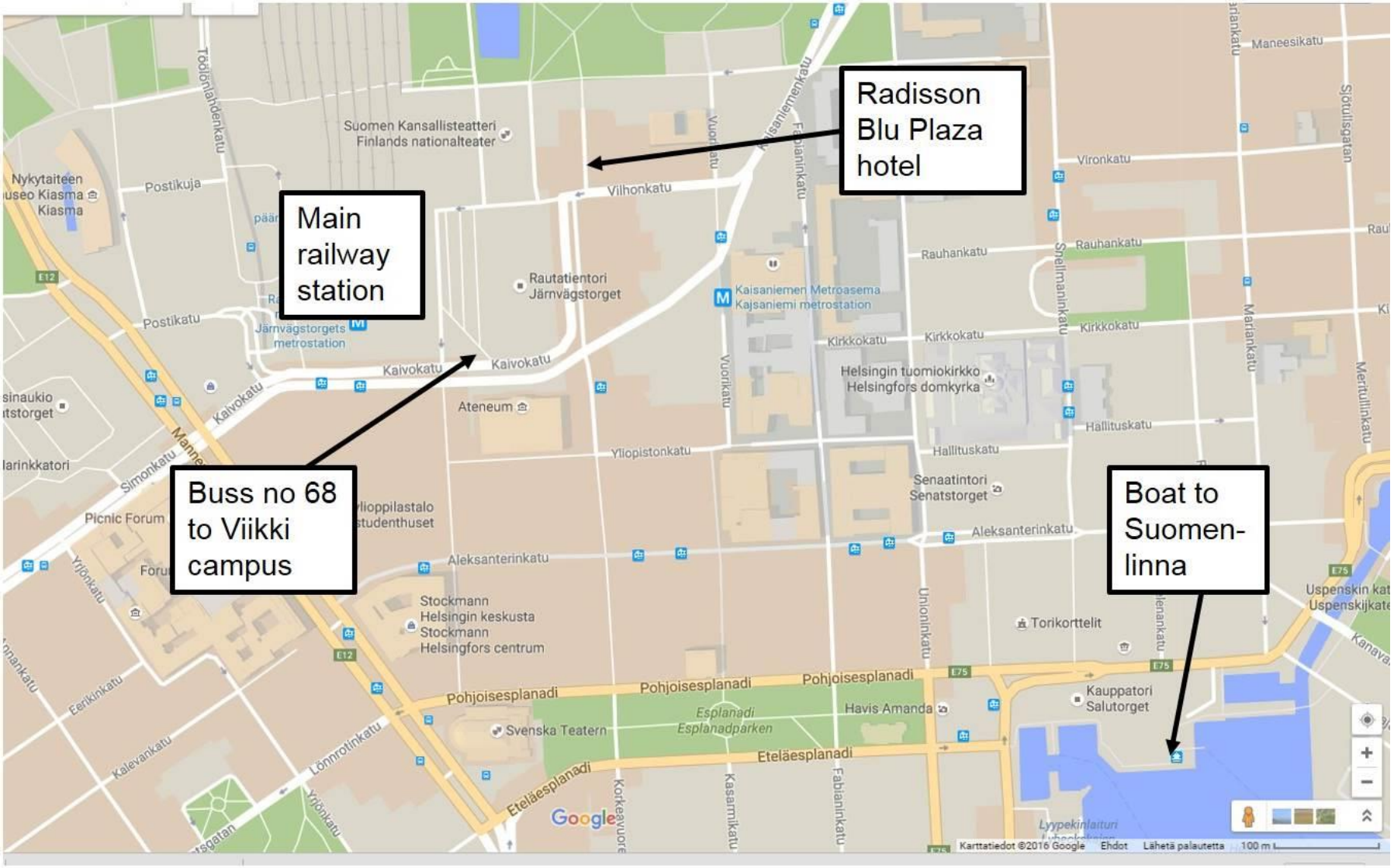
Novel *Dickeya* species - again?

ALIČ Š[1,2], VAN GIJSEGEM F[3], NAGLIČ T[1,4], PÉDRON J[3], TUŠEK-ŽNIDARIČ M[1], , RAVNIKAR M[1,4], DREO T[1,4]*

[1]*National Institute of Biology, Dept. of Biotechnology and Systems Biology, Slovenia,*
[2]*Jožef Stefan International Postgraduate SchoolSlovenia,*
[3]*UPMC Univ Paris 06, INRA, IEES Paris (Institute of Ecology and Environmental Sciences), France*
[4]*COBIK - Centre of Excellence for Biosensors, Instrumentation and Process Control, Centre for Biotechnology,Slovenia*
*Corresponding author: tanja.dreo@nib.si

Bacteria isolated from diseased tissues of Phalaenopsis orchids from a commercial production site were identified as *Dickeya* spp.. Comparative genomic study of genomes of the two selected isolates B16 and S1 (Alič *et al*., 2015) and publicly available *Dickeya* genomes identified the isolates as a putative new species within genus *Dickeya*, along with MK 7 and NCCPB 3274 strains. Based on partial sequencing of fliC the isolates are similar to *Dickeya* isolates from Asia (Suharjo *et al*., 2014) indicating that the new species may be widespread in nature. Strains of the putative new species show unique carbon assimilation profile and high aggressiveness on chicory leaves. Isolates B16 and S1 were able to systemically colonize potato plants.

Helsinki city center



Viikki campus

The meeting will take place in Latokartanonkaari 5, 3rd floor at
Department of Agricultural Sciences
The house is also called C building

Buss stop of buss no 68 by the gorilla and blue round library building

Entrances to Latokartanonkaari 5

All entrances marked with a star

Euphresco III
Dickeya / Pectobacterium Workshop

1st and 2nd November 2017
SASA, Edinburgh, Scotland, UK

Programme & Abstracts

Euphresco III

Dickeya/Pectobacterium Workshop

SASA, Edinburgh, Scotland, UK

1st & 2nd November, 2017

Wednesday 1st November

08:50	Meet in Hotel foyer for optional tour. (*See additional information*)
09:30-11:00	Tour of Edinburgh vaults
11:15	Bus back to SASA from Old Town (private bus)
11:30	Pick up from hotel for those who didn't attend tour
12pm	Arrive at SASA for Lunch
12:45-13:00	Introduction – Gerry Saddler

Session 1: Surveys (Chair, Ian Toth)

13:00-13:20	Greig Cahill, SASA, Edinburgh: Blackleg in Scotland - The current picture
13:20-13:40	Yeshitila Degefu, National Resources Institute Finland (Luke): Observation on latent occurrence and co-occurrence of *Dickeya* and *Pectobacterium* species in potato tubers.
13:40-14:00	Jacquie van der Waals, University of Pretoria: Detection of soft rotting *Enterobacteriaceae* in irrigation water used in potato production systems in South Africa.
14:00-14:20	Yves le Hingrat, FN3PT, Paris: The national research program on blackleg disease in France: what are the main outcomes?
14:20-14:40	Rachel Mann, AgriBio, Victoria: *Dickeya dianthicola* in Australia
14:40-15:00	Gary Secor, NDSU, Fargo: Updates on Detection and Spread of *Dickeya* in the US.
15:00-15:30	Tea/Coffee

Session II: Genomics (Chair, Gerry Saddler)

15:30-15:50	Sonia Humphris/Ian Toth, JHI, Dundee: An investigation into the interplay between metabolism and pathogenicity in *Pectobacterium* through the Entner-Douderoff pathway.
15:50-16:10	Jakub Fikowicz Krosko, University of Gdansk: Fast and reliable screening system to preselect candidate *Dickeya solani* Tn5 mutants in plant tissue-induced genes.
16:10-16:30	Frederique van Gijsegem, INRA-UPMC, Paris: New insights into the genomic diversity of *Dickeya solani* and *Dickeya dianthicola.*
16:30-16:50	Leighton Pritchard, JHI Dundee: *Pectobacterium atrosepticum* subspecies structure in Scotland.
16:50-17:10	Ewa Łojkowska, University of Gdansk: Pangenome of plant pathogenic bacterium *Pectobacterium parmentieri.*
17:10-17:30	Discussion and round up
17:30	Bus leaves for Haymarket Hub Hotel
19:30	Dinner at First Coast, Dalry Road (see Additional Information)

Thursday 2nd November

08:00 Pick up from Hotel

Session III: Diagnostics (Chair, Ewa Łojkowska)

09:00-09:20 Mirjam Prinz, Bavarian State Research Center for Agriculture, Freising: Development of a multiplex assay for blackleg bacteria directly applied to potato tuber sap.

09:20-09:40 Minna Pirhonen, University of Helsinki: Potato dextrose agar as an easy way to identify *Dickeya solani*.

09:40-10:00 Jeremy Cigna, SIPRE, France: Rapid method to identify pectinolytic bacteria isolated from blackleg symptoms in potato fields: Development and application of the "gapA" tool.

Session IV: Epidemiology and Control (Chair, Minna Pirhonen)

10:00-10:20 Jan van der Wolf, Wageningen UR: Suppressiveness of potato against *Dickeya solani*

10:20-10:40 Amélie Beury, SIPRE, France: Introduction of two biocontrol bacteria into potato plant rhizosphere to prevent latent contamination by pectinolytic bacteria naturally present in soil and blackleg symptom development.

10:40-11:00 Tea/Coffee

11:00-11:20 Ivette Acuña, INIA, Chile: Potato seed treatment as a part of an integrated management of blackleg and soft rot in potato.

11:20-11:40 Brice Dupuis, Agroscope: Occurrence and aggressiveness of *Pectobacterium* and *Dickeya* strains in Switzerland.

11:40-12:00 Robert Vreeburg, NAK, Emmeloord: Initial infection of seed potato lots with *P. brasiliense.*

12:00-12:20 Doretta Boomsma, HZPC Research B.V. Netherlands: Evaluation of the field tolerance of potato cultivars to single and mixed infections of different *Dickeya*/*Pectobacterium* species.

12:20-12:40 Alison Blackwell, APS Biocontrol Ltd: Developing Bacteriophage for the Management of Blackleg.

12:40-13:25 Lunch

Session V: Plant-Pathogen Interactions (Chair, Jan van der Wolf)

13:25 – 13:45 Marie-Anne Barny, Institute of Ecology and Environmental Science, Paris: Phenotypic characterization on plant of soft rot enterobacteria isolated outside the plant context.

13:45 – 14:05 Guy Condemine, University of Lyon: Identification of new *Dickeya dadantii* virulence factors: a Tn-seq approach.

14:05 – 14:25 Vladimir Gorshkov, Kazan Institute of Biochemistry and Biophysics, Russia: Stealth and brute force behaviour of *Pectobacterium atrosepticum* inside the plant.

14:25 - 14:45 Marta Potrykus, University of Gdansk: The influence of two quorum sensing systems on the phenotypic features of *Dickeya solani* exhibiting different virulence levels.

14:45-15:05 Iris Yedidia, Volcani Centre, Israel: Interkingdom signalling: interference of plant derived small molecules with bacterial communication and virulence.

15:05-15:25 Patrice de Werra, Bern University of Applied Sciences: Transmission of blackleg symptoms and latent infection from plant to plant under greenhouse conditions.

15:25-16:00 Tea/Coffee and final discussion

16:00 Close

16:30 Bus departs to hotel
Taxis to airport

Abstracts

"Blackleg in Scotland: The current picture"

Greig Cahill, Gerry Saddler
Science and Advice for Scottish Agriculture (SASA), Edinburgh, Scotland (UK)
Email: greig.cahill@sasa.gsi.gov.uk & gerry.saddler@sasa.gsi.gov.uk

Blackleg continues to be the principal cause of downgradings and rejections of seed crops in Scotland. Recent changes to seed certification which introduced a compulsory third inspection under some circumstances will be discussed. In Scotland, unlike other parts of Europe, *Pectobacterium atrosepticum* remains the predominant pathogen causing infection, and since 2010 annual surveys of tubers and crops have confirmed the absence of *Dickeya* spp. Results from these surveys will be presented, including the status of *Pectobacterium wasabiae* and *Pectobacterium carotovorum* subsp *brasiliense* present in Scotland. The results of a pilot project testing Pre-Basic crops for latent *Pba* infection will also be discussed.

"Observation on latent occurrence and co-occurrence of *Dickeya* and *Pectobacterium species in potato tubers*"

Yeshitila Degefu, Natural Resources Institute Finland (Luke), Green Technology Unit, Paavo Havaksen tie 3, P.O.Box 413, FI-90014 University of Oulu, Finland. yeshitila.degefu@luke.fi

The *Enterobacteriaceae* is a large family of Gram-Negative bacteria that includes, in addition to many harmless symbionts, the more familiar human bacterial pathogens and the bacteria in the genera *Dickeya* and *Pectobacterium* which cause blackleg and soft rot in potato. *Dickeya solani, Dickeya dianthicola, Pectobacterium atrosepticum, Pectobacterium carotovorum subsp., Pectobacterium brasiliense* and *Pectobacterium wasabiae* are the currently known *enterobacteriacae* causing blackleg and soft rot in potato in Europe and globally. The Bacteria latently present in the tuber are the main sources of infection and it is often observed that seed lots and the potato plant could be contaminated by one or more of these species. Since 2005 the lab at the now Natural Resources Institute Finland (Luke) has been providing seed tuber testing (diagnostic) services to potato growers and seed potato production and distribution companies in Finland besides the blackleg and soft rot research. The test results have been carefully documented. This presentation highlights the results of the thirteen years of seed testing and summarizes the occurrence and co-occurrence of the bacterial species during latent survival in the tubers. Which species appear happy together and which ones, according to the statistics of occurrence, cohabit less frequently or even did not appear to share the tuber niche? The presentation is based only on observation of analysis test results of nearly 1500 samples over a thirteen year period and it is simply preliminary data which requires further investigation *in vitro* and *in planta*. At this time where researchers are scratching the surfaces for possible control measures of blackleg and soft rot in potato, it might be useful to get some insight about the meaning of the diversity and interactions among these threatening bacterial pathogens of potato. Could it be possible that *Dickeya* and *Pectobacterium* species nourish their own controls?

"Detection of soft rotting *Enterobacteriaceae* in irrigation water used in potato production systems in South Africa"

van der Waals, J.E., Laughton, N., Chidamba, L. & Korsten, L.
Department of Plant and Soil Sciences, University of Pretoria, Private Bag X20, Hatfield, 0028

In South Africa, up to 85% of potatoes are produced under irrigation; hence the need to evaluate the prevalence of *Pectobacterium* and *Dickeya* species in agricultural water sources for more effective disease management strategies. A total of 104 water samples were collected from 50 sampling points including dams (20), boreholes (3), rivers (6) and overhead irrigation systems (21) in seven potato growing regions of South Africa. Crystal violet pectate selective culturing of SREs from enriched water samples, isolation and PCR identification of the various species, detected the pathogens in water systems used for irrigation in all of the sampled regions except one. Species which are known to cause diseases in potatoes including *Dickeya* spp., *Pectobacterium carotovorum* subsp. *brasiliense*, *Pectobacterium carotovorum* subsp.

carotovorum, Pectobacterium atrosepticum and *Pectobacterium parmentieri* were identified from the water sources sampled. Preliminary pathogenicity evaluation of the isolates showed that some can cause soft rot of tuber slices. This is an important finding for the South African potato industry to profile the disease-causing potential of agricultural irrigation water.

"The national research program on blackleg disease in France: what are the main outcomes?

Hélias Valérie[1,2], Chawki Khaoula[1,2], Quêtu-Laurent Angélique[1,2], Andrivon Didier[2], Le Hingrat Yves[1]

[1]FN3PT/RD3PT, French Federation of Seed Potato Growers, 43-45 rue de Naples, 75008 Paris, France
[2]INRA, UMR1349 IGEPP, 35653 Le Rheu Cedex, France

Since 2003 the FN3PT has been leading a research program on *Pectobacterium* and *Dickeya* in collaboration with INRA. The four themes of the program are diversity, detection, epidemiology and control of the disease.

Field surveys, together with diversity studies of the resulting strain collections, showed that blackleg symptoms are associated with a much larger range of species than the one *P. atrosepticum* which was historically almost exclusively held responsible for these symptoms in Europe. A collaborative project with the CFPB-CIRM was developed for analyzing more deeply the diversity and the phylogeny of *Pectobacterium* using MLSA. Pectinolytic bacteria identification was also performed using gapA (presentation of Cigna *et al*.).

Consequently, the availability of high performance, fast and reliable detection tools for all members of this genus is of utmost importance for both seed certification and disease control. Recent evaluations of the performance (sensitivity, specificity and detection thresholds) of several published real-time assays contribute to that objective.

Molecular detection methods were used to assess bacterial contamination on tubers during a French-Swiss concerted action (FN3PT-Bern Univ. Plant Sciences). One of the objectives of the project was to find out if evaluations of latent infections improve the prediction of subsequent disease development risk.

In parallel with the development of biocontrol strategies, (see Munier *et al*. presentation) the use of genetic resistance constitutes an interesting prospect in the control of blackleg disease, which currently relies only on prophylactic measures. Over the last decade, FN3PT has observed changes in the bacterial complex responsible for soft-rot and blackleg. These changes have led to the evaluation of 24 clones (CRBBraCySol) which were identified by INRA in three Solanum species in the late 1990's. Previous studies demonstrated that these clones have a resistance to *P. atrosepticum* but no information existed up to now on resistance levels of genetic resources for these newly identified bacterial groups. The recent outcomes of the different studies will be presented. "

"An outbreak of *Dickeya dianthicola* in potatoes in Australia 2017"

Rachel Mann[1,2], Monica Kehoe[3], Elisse Nogarotto[1,2], Dominie Wright[3], Brenda Coutts[3], Brendan Rodoni[1,2]

[1]AgriBio, Agriculture Victoria Research, Bundoora, VIC, Australia
[2]Plant Biosecurity Cooperative Research Centre, Canberra, ACT, Australia
[3]Department of Primary Industries and Regional Development, South Perth, Western Australia

In May 2017, uncharacteristic blackleg symptoms were observed by an inspector in potato crops in Western Australia. The causative organism was identified as *Dickeya dianthicola* at the Department of Primary Industries and Regional Development and confirmed at secondary laboratories in New South Wales and the Northern Territory, Australia. This is the first time *D. dianthicola* has been detected in Australia. An immediate incident response was put in place by the Western Australia government with surveillance and tracing activities implemented. *D. dianthicola* was detected in seed potatoes, dormant dahlia tubers and freesia bulbs and tracing of these hosts occurred to and from infected properties. This presentation will outline the outbreak, with a focus on the diagnostics.

"Updates on Detection and Spread of *Dickeya* in the US"

Gary Secor, Viviana Rivera-Varas and Blake Greiner. Department of Plant Pathology, North Dakota State University, Fargo, ND 58102 USA

This presentation will provide updates on detection and spread of *Dickeya* in potatoes. *Dickeya dianthicola* is an important component of the potato soft rot complex in the US causing serious field stand loss. Warmer temperatures are necessary for expression of *Dickeya* symptoms, but infection is usually symptomless in seed potatoes produced in cool climate areas. Consequently, it is necessary to test seed tubers for latent *Dickeya* infection using a PCR test. *Dickeya* was detected in the stem end, the tuber shoulder, peel strip, and sprout, but the peel strip has the highest frequency of detection compared with the other tissues. Lenticels may play an important role as source of infection in seed tubers, and PCR testing both stem end core and peel samples may be necessary to maximize latent *Dickeya* detection in seed lots. Field trials failed to show spread of *Dickeya* during seed handling and cutting operations. An irrigated field trial was conducted to detect the spread of *Dickeya* from infected seed to adjacent plants and tubers during the growing season. Low amounts of seed decay and blackleg were observed in adjacent plants and *Dickeya* was detected in only a few of the tuber samples by post-harvest PCR testing.

"An investigation into the interplay between metabolism and pathogenicity in *Pectobacterium* through the Entner-Douderoff Pathway"

Huan Wang[1,2], Shuo Du[1], Yujie,Wang[1], Jiaqin Fan[1] Sonia Humphris[2] and Ian Toth[2]

[1]Nanjing Agricultural University, Nanjing, 210095, China
[2]The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK

The Entner-Douderoff (ED) pathway is one of the alternative glycolytic pathways in prokaryotic glucose metabolism. Our previous work has shown that eda (encoding 2-keto-3-deoxy-gluconate-6P aldolase), which is a key enzyme in the ED pathway and the breakdown of pectic substances in the plant, is required for full virulence in *P. carotovorum* subsp. *carotovorum* (*Pcc*) strain PccS1. Comparison of genomes showed PccS1 possesses an incomplete ED pathway (missing gene edd, which encodes enzyme 6-phosphogluconate dehydratase), while *P. atrosepticum* (*Pba*) has a complete pathway. A deletion mutant of eda in *Pba* showed attenuated virulence and was unable to effectively degrade pectin, whereas a deletion mutant of edd had no effect on virulence or pectinase activity. Our results show that eda plays an important role in the virulence of *Pba* but this appears to be through the degradation of pectin rather than through the ED pathway

"Fast and reliable screening system to preselect candidate *Dickeya solani* Tn5 mutants in plant tissue-induced genes"

Jakub Fikowicz-Krośko[1], Robert Czajkowski[1]
[1]Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk

In this study we presented a simple and fast screening method to preselect candidate *Dickeya solani* Tn5 mutants which carry random transposon insertions. The Tn5 mutants are generated using mini-Tn5 transposon carrying inducible promoterless gusA reporter gene (β-glucuronidase) and are screened for the β-*glucuronidase* positive phenotypes under non-inductive conditions. The bacterial mutants negative in the first screen are then screened for β-*glucuronidase* phenotypes in the presence of plant tissues. In the study we have used stems, leaves and roots of *Solanum tuberosum* and *Solanum dulcamara*. The mutants which are positive in the screen with plant tissues are selected for sequencing of the Tn5 insertion sites directly from bacterial genome. The described method is faster and cost-effective in comparison to standard IVET or expression microarrays and RNAseq. It allows to generate a number of ready-to-use Tn5 mutants revealing up-regulation by plant tissue which can be later selected for further studies.

"New insights into the genomic diversity of *Dickeya solani* and *Dickeya dianthicola*"

Jacques Pédron[1], Géraldine Taghouty[2], Perrine Portier[2] and Frédérique Van Gijsegem[1]
[1]iEES Paris (Institute of Ecology and Environmental Sciences), INRA-UPMC Université Paris 06, UMR 1392, F75005, Paris, France
[2]CIRM-CFBP, INRA – IRHS, F-49071 Beaucouzé, France

D. solani and *D. dianthicola* were mainly isolated from potato and various ornamentals with some *D. dianthicola* occurrences also in chicory and tomato. A recent reclassification of all former *Erwinia chrysanthemi* strains present in the CFBP collection into the different *Dickeya* species allowed the identification of a new *D. solani* strain that was isolated from tomato in Guadeloupe. On the other, differences in aggressiveness on potato were reported for various isolates in both species. From the genomic point of view, *D. solani* was shown to be mainly clonal while *D. dianthicola* appeared to be more diverse from MLSA analyses. We undertook comparative genomic analyses to unravel the possible links between genomic traits and aggressiveness and/or host of isolation. Both species share more than 3,000 genes including most of the genes that were shown to be involved in virulence in the *D. dadantii* 3937 model strain. Intraspecific diversities will be discussed.

"*Pectobacterium atrosepticum* subspecies structure in Scotland"

Leighton Pritchard, Sonia Humphris, Ian K Toth
James Hutton Institute (Dundee) Errol Road, Invergowrie, Perth and Kinross, Scotland, DD2 5DA

We obtained complete genome sequences for ≈50 *P. atrosepticum (Pba*) isolates that were either previously sampled by SASA as part of a long-term study in Scotland, or were derived from a field trial at the James Hutton Institute. The sequenced SASA isolates represent a restricted geographical area, and were isolated in the period 2009-2015. Comparative genomic analyses of the assembled, annotated isolates were able to distinguish between phenotypically-similar *Pba* and non-*Pba* isolates, and indicated considerable genomic variation within *Pba*. The observed genomic variation was divisible into four clearly separated subspecies groups, divisible by ANI, SNP trees and core/accessory gene content. No clear geographic pattern in subspecies incidence across Scotland was seen, and the genomic variation of these Scottish isolates was comparable to the genomic variation of isolates obtained from Australasia, the Americas, Asia, and Europe.

"Pangenome of plant pathogenic bacterium *Pectobacterium parmentieri*

Ewa Łojkowska[1], Sabina Zoledowska[1], Agata Motyka[1], Wojciech Sledz[1] and Alessio Mengoni[2]

[1]Department of Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, 58 Abrahama Street, 80 307, Gdansk, Poland
[2]Department of Biology, University of Florence, via Madonna del Piano 6, 50019 Sesto Fiorentino, Florence, Italy

Pectobacterium parmentieri and *Dickeya solani* are newly established species within *Pectobacteriaceae* family. Interestingly, we observe significant phenotypic differences in terms of virulence factors production and ability to macerate potato tissue not only among genomically heterogenous strains of *P. parmentieri* but also among genomically homogenous *D. solani* strains. In order to elucidate the source of these differences, we sequenced several *P. parmentieri*. A pangenome study was performed on 8 genomes of *P. parmentieri*. The pangenome structure of the species *P. parmentiri* was compared with those of *D. solani* species. *P. parmentieri pangenome* includes 3788 core genes, 1201 accessory genes, and 1625 unique genes. Analysis of *D. solani* pangenome indicate that number of *D. solani* core genes is very similar and contain 3756 core genes, however, the accessory and the unique genome is much smaller and include 430 and 130, respectively. We were able to determine the presence of all genes encoding well-known virulence factors in core genomes of both species.

"Development of a multiplex assay for blackleg bacteria directly applied to potato tuber sap"

Mirjam Prinz, A. Kellermann, G. Bauch
Bayerische Landesanstalt für Landwirtschaft IPZ6a Am Gereuth 8, 85354 Freising

Testing for blackleg bacteria on seed potatoes is economically very important due to massive crop loss in the field and potentially high influence of latent infection on exported tubers.

Current methods to detect different species of bacteria are conventional PCR and quantitative real-time PCR that are applied to DNA extracts of potato tuber sap. However, extraction of DNA is time consuming and costly and thus impractical to use for high throughput screening of seed potatoes.

Therefore, we develop a multiplex real-time qPCR assay that is applied directly on diluted potato tuber sap (DiRT-qPCR). In my talk, I will present the latest status of the developed method including the optimization of the multiplex reaction and treatment of potato tuber sap.

References

Stammler et al., DiRT-qPCR for molecular biological detection of RNA encoded viruses. Acta Horticulturae in press.
Czajkowski et al. 2011. Control of blackleg and tuber soft rot of potato caused by Pectobacterium and Dickeya species: a review. Plant pathology 60, 6, 999-1013."

"Potato dextrose agar as an easy way to identify *Dickeya solani*

Minna Pirhonen, University of Helsinki

Isolation and identification of bacteria from diseased plant tissues is a time consuming task. When *Dickeya solani* grows as pure culture on potato dextrose agar (PDA), it grows as distinctive wrinkled and brownish red colonies, enabling fast and efficient identification. Streaking bacterial pure cultures on PDA reveals if the isolate belongs to *D. solani* species. Furthermore, spreading bacteria directly from diseased tissue on PDA plates may work as a rapid way to isolate and identify *D. solani* directly from the plant tissue, at least when high concentration of *D. solani* cells are present in the plant tissue. Not all PDA work equally well, the best is the PDA from Biokar.

"Rapid method to identify pectinolytic bacteria isolated from blackleg symptoms in potato fields: Development and application of the "gapA" tool

Cigna Jérémy[1-2], Dewaegeneire Pauline[1], Beury-Cirou Amélie[1], Gobert Virginie[1] and Faure Denis[2]

[1]Seeds Innovation Protection Research Environment (SIPRE), rue des champs Potez, 62217 Achicourt, FRANCE
[2]CNRS-I2BC, ANR-15-CE21-0003, 1 avenue de la terrasse, 91198 Gif-sur-Yvette, FRANCE

SIPRE (Seeds Innovation Protection Research Environment) is mandated by the CNPPT (Comité Nord Plants de Pomme de Terre, a seed potato growers association located in North of France) to bring concrete solutions to seed potato growers problems. SIPRE develops biocontrol strategies against *Pectobacterium* and *Dickeya* in collaboration with the FN3PT/RD3PT (French Federation of Seed Potato Growers) with the national research program on blackleg disease in France (cf. presentation of Hélias *et al*.) and with the CNRS-I2BC (cf. presentation of Munier *et al*.).

In order to test our biocontrol strategies on current pathogens, we isolated near to 700 pathogenic strains from blackleg symptoms in potato fields over the last 5 years.

To ensure the characterization of all pectinolytic strains isolated, we have developed a rapid and accurate molecular method focused on gapA gene. Diversity and phylogeny of *Pectobacterium* was also performed using MLSA (cf. presentation of Hélias *et al*.).

Firstly, an alignment of gapA genes retrieved from genomes publicly available was performed and specific signature-nucleotides were searched. A phylogenic tree has confirmed the identity of each species of *Pectobacterium* and *Dickeya*, and validated the approach.

Then, we developed a single PCR-sequencing "gapA" method in order to characterize quickly the 700 soft rot pectinolytic bacteria isolated in blackleg symptoms of potato fields. Results will be presented.
Finally, we will test the biocontrol activity of protection strains previously studied on a panel of contemporary pathogens identified with gapA phylogeny.

"Suppressiveness of potato against *Dickeya solani*"

*Jan van der Wolf, Claudia Coipan, Pieter Kastelein, Marjon Krijger, Jolanda Tom, Natasja Riksen, Els Nijhuis, Sven Warris en Jenny Fokkema**

Wageningen UR, Postbus 16, 6700 AA Wageningen, T 0317-480598, E Jan.vanderWolf@wur.nl
**NAO, Van Stolkweg 31, 2585 JN Den Haag*

In two years field experiments, potato seed lots of two different cultivars (ca. 20 per cultivar per year), originating from different locations in the Netherlands were planted in the same field in randomized blocks. The natural background of blackleg causing bacteria in the selected seed lots was low. Prior to planting, seed lots were vacuum-infiltrated with *Dickeya solani* (10^6 cfu/ml). Large differences in blackleg incidences were found between seed lots of the same cultivar within a year, which can only be explained by differences in suppressiveness of the seed lots against *D. solani*, as the genetic background of the seed lots was similar, lots were planted in the same field and were infected with the same densities of the pathogen.
To address the question which factor determines suppressiveness, seed lots were analysed for the mineral composition, dry weight and the bacterial microbiome. A weak relation was found between suppressiveness and some physico-chemical properties. We also found a relation between suppressiveness and the presence of specific bacterial taxons. To study the potential involvement, bacteria belonging to these taxons were isolated from seed lots, characterized and analysed for (in vitro) antagonism against soft rot *Pectobacteriaceae*.

The research was financed by the Ministry of Economic Affairs and by Dutch potato breeding companies, under the coordination of the Dutch Potato Organization (NAO)

"Introduction of two biocontrol bacteria in potato plant rhizosphere to prevent latent contamination by pectinolytic bacteria naturally present in soil and blackleg symptoms development"

Euphrasie Munier[1], Pauline Dewaegeneire[1], Jérémy Cigna[1], Julie Bartier[1], Valérie Hélias[2], Denis Faure[3] and Amélie Beury[1]

[1] SIPRE, Seeds Innovation Protection Research Environment, Rue des champs Potez, 62217 Achicourt
[2]FN3PT/RD3PT, UMR 1349 IGEPP INRA, Agrocampus Ouest Rennes, Université Rennes, 35653 Le Rheu, France
[3]CNRS, Institut de Biologie Intégrative de la Cellule UMR 9198 CEA, Université Paris Sud USC 1358 INRA, CNRS, Université Paris Sud, CEA, 1 Avenue de la terrasse, 91 198 Gif-sur-Yvette, France

Two combined biocontrol strategies for potato plants were developed in order to control and maintain at a low level soil-borne pectinolytic bacteria naturally present in the soil microbiota and to minimize tuber infections. These strategies rely on the direct application in-furrow of two bacterial biocontrol agents; including *Pseudomonas putida* A14H7, exhibiting antibiosis activity (Raoul des Essarts *et al*, 2016), and *Rhodococcus erythropolis* R138 (Cirou *et al*, 2007) exhibiting a quorum-quenching activity. *R. erythropolis* R138 is able to assimilate quorum-sensing signal molecules such as N-acyl homoserine lactones involved in virulence regulation in pectinolytic bacteria. The effect of this combined treatment on the acquisition of soil-borne pectinolytic bacteria by tubers and blackleg symptoms expression on plants was evaluated. The two biocontrol agents were monitored over time in the soil rhizosphere during the growing period to

ensure that they were maintained. The field trials were conducted over three successive field generations. The results showed either a non-acquisition of pectinolytic bacteria in tubers or a decrease of blackleg symptoms in potato plants grown from treated soil. Our data suggest that this biocontrol strategy could provide significant protection of potato plants against pectinolytic bacteria in field conditions.

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Cirou et al, 2007 Environ. Microbiol. 9:1511-1522.

"Potato seed treatment as a part of an Integrated management of blackleg and soft rot in potato"

Acuña, I[1].; Sandoval, C[1].; Gutiérrez, M[2].; Bermúdez[1], A.; Araya[1], M. and S. Mancilla[1]

[1]Instituto de Investigaciones Agropecuarias, INIA Chile.
[2]Laboratorio Regional Osorno, Servicio Agrícola y Ganadero SAG Chile
E-mail: iacuna@inia.cl

Seed decay, soft rot and blackleg are considered important diseases in the potato production system in Chile. A survey done by INIA shows that 13.5% and 64.8% of the strains causing the disease are *P. atrosepticum* and *P. c.* subsp*. carotovorum* respectively. Additionally, in the last years *Dickeya dadantii* also has been described in potatoes. Due to the importance of this problem, the Chilean Innovation Agency (FIA) has financed a new research grant. One of the objectives of this project is to develop an integrated management strategy for potato bacterial diseases, based on a real time PCR assay as a seed rot potential to determine the sanitary risk and preventive measures. During the season 2016-17 a storage experiment was performed to evaluate the efficacy of seed potato treatment to manage seed rot potential and latent infection. Results will be discussed.

"Occurrence and aggressiveness of *Pectobacterium* and *Dickeya* strains in Switzerland

B. Dupuis[1], Ch. Sagnier[1], I. Kellenberger[1], S. Schaerer[1]
[1]Agroscope, Nyon, Switzerland

Summary: In Switzerland, blackleg is the primary ground for rejection of potato seed lots after field inspection. A new survey of *Dickeya* and *Pectobacterium* strains was carried out in Switzerland in 2017. Samples of plants with blackleg symptoms were collected in the post-harvest grow-out fields. In total, 62 plots were sampled, corresponding to 62 seed potato fields harvested in 2016. The samples were analyzed by PCR to identify the pathogens responsible for the infection. In addition, a greenhouse trial was undertaken to assess the aggressiveness of *Pectobacterium carotovorum* subsp *carotovorum*, *Pectobacterium carotovorum* subsp *brasiliense*, *Pectobacterium wasabiae*, *Pectobacterium atrosepticum*, *Dickeya dianthicola* and *Dickeya solani*. The aggressiveness of two isolates per (sub)specie was tested. Twenty tubers of cv. Désirée were inoculated by soaking in a bacterial suspensions of each isolate (10^6 cfu/ml). The tubers were then planted in pots containing 5 L of soil and maintained in a greenhouse at 25°C and 70% of relative humidity. The expression of the symptoms was followed for 35 days after emergence, and the progeny tubers were weighted after harvest. The survey revealed that *Pectobacterium carotovorum* subsp *brasiliense* was the most prevalent bacteria, with 51.6% of occurrence in the plots, followed by *Pectobacterium carotovorum* subsp *carotovorum* and *Pectobacterium wasabiae* with both 35.5% of occurrence, by *Dickeya dianthicola* with 11.3% of occurrence, and finally by *Dickeya solani* with 4.8% of occurrence. *Pectobacterium atrosepticum* was never detected. Mixed infections were found in 27.4% of the plots, with 17.7% of plots infected by two distinct (sub)species and 9.7% infected by three distinct (sub)species. The greenhouse trial revealed that *Dickeya solani* was more aggressive than the other species and also that no symptoms were expressed by the plants inoculated with *Pectobacterium carotovorum* subsp *carotovorum.* All bacteria species affected the yield, which was most reduced after inoculation with *Dickeya solani*.

Acknowledgments: The authors want to thank Peter Frei, Werner Wild, Maud Tallant, Gaël Droz and Loris Socchi for their technical support.

"Initial infection of seed potato lots with *P. brasiliense*"

Robert Vreeburg, NAK (Dutch general inspection service), Randweg 14, 8304 AS Emmeloord

The increase in the fraction of samples of Dutch seed potato that is latently infected with *P. brasiliense* with advancing generation, can be modelled by assuming a yearly chance of new latent infections (P) and a chance for an infection to disappear (Q). Fitting this model on the data of *P. brasiliense* gave estimates for P and Q being ~30% and ~10%.

We try to find the infection route that can explain the large infection chance of *P. brasiliense*. Preliminary data indicates that (aerosols from) surface water is not a likely source. The methodology for detecting *P. brasiliense* in insects proved to be very insensitive, not allowing to collect quantitative data. Several bulks of insects tested positive by PCR for *P. brasiliense*. Work is in progress to isolate the bacteria and confirm their identity.

"Evaluation of the field tolerance of potato cultivars to single and mixed infections of different *Dickeya/Pectobacterium* species

Doretta Boomsma: HZPC Research B.V. Roptawei 4, 9123 JB Metslawier, The Netherlands

In 2015 and 2016 field trials were carried out in the Netherlands to evaluate the field tolerance of about 40 potato varieties to single and mixed infections of 3 different *Dickeya/Pectobacterium* species. Per variety minitubers were vacuum infiltrated with only water (control), *Dickeya solani* (Ds), *Pectobacterium wasabiae* (*Pw*), *P. carotovorum* subsp. *brasiliense* (*Pcb*), and mixtures of *Ds+Pw, Ds+Pcb, Pw+Pcb*, so 7 treatments per variety. Per variety and per treatment 12 tubers were planted per plot in 4 replications. All plots were assessed for plant emergence and symptom development in time. After harvest the yield (kg) and the number of tubers/plot were determined. All daughter tubers were divided in 8 equal samples and, after enrichment, tested the presence of *Ds, Pw, Pcb* and *Pa* by Taqman PCR. It was shown that disease symptom expression has random patterns that are mostly correlated to the factors year and lot/variety but not to treatment

"Developing Bacteriophage for the Management of Blackleg"

Alison Blackwell, APS Biocontrol Ltd. Prospect Business Centre, Gemini Crescent, Dundee Technology Park, Dundee DD2 1TY

Blackleg is a major cause of seed potato downgrading and rejections in northern Europe, together with production losses from associated tuber soft rot. Blackleg inoculum (*Pectobacterium* & *Dickeya*) is transferred and multiplied between successive field generations and problems in seed multiplication create issues for the whole of the supply chain, with up to 30% of ware crops affected from planting infected seed stocks and post-harvest rots affecting the processing, fresh sales, wholesale and pre-packing industries. The challenge addressed in this study is to develop an innovative, sustainable bactericide treatment applicable across the industry, to both reduce seed tuber infection levels and protect against wastage from tuber soft rots downstream. Treatments under development are based on formulations of naturally-occurring, highly-specific and environmentally-acceptable agents (bacteriophage); data will be presented to support their incorporation into a novel biological-control agent, with the potential to have a significant impact on the UK industry, as well as to provide a marketing advantage to seed-potato exports over major competitors.

"Phenotypic characterization on plant of soft rot enterobacteria isolated outside the plant context"

Bertrand C[1], Munier E[2], Lecomte A[1], Beury A[2], Berge O[3] and Barny Ma[1]

[1]Institute of Ecology and Environmental Science-Paris
University Pierre and Marie Curie - 4 place Jussieu - 75252 PARIS cedex 05
[2]SIPRE, Rue des champs Potez, 62217 Achicourt
[3]INRA,Plant Pathology Research Unit, 84143 Montfavet, France

We previously isolated a large panel of Soft Rot Enterobacteria (SRE) outside of the plant context. A subset of these isolates was tested on a plant panel to assess their potential aggressiveness on plants and compared with type strains isolated from various host plants. The panel of plant tested was chosen among the plants where both *Dickeya* and *Pectobacterium* were isolated according to the literature (Ma *et al*, 2007). Furthermore, for potato, both tubers and plants were tested while two developmental stages were tested for cantaloupe. The inoculation procedures and results obtained will be presented.

"Identification of *Dickeya dadantii* new virulence factors : a Tn-seq approach

Kevin Royet[1], Nicolas Parisot[2], Agnès Rodrigue[1], Erwan Gueguen[1] and Guy Condemine[1]

[1]Université Lyon 1, INSA de Lyon, CNRS UMR5240 MAP, Villeurbanne, France
[2]INSA de Lyon, INRA, UMR203 BF2I, Villeurbanne, France

Identification of virulence factor genes by testing individual mutants on plants is a very time and labour consuming method. Development of high throughput sequencing methods allows the test of a collection of hundreds of thousands transposon mutants in a single experiment by the Tn-seq approach. This method allows the detection of mutants with reduced but also increased fitness in the plant. We used this method to identify new virulence factors of *Dickeya dadantii* on chicory. Virulence factors identified differed from those previously known since diffusible ones (secreted enzymes, siderophores or metabolites) could not be detected by this screen. In addition to genes encoding proteins of unknown function that could be new virulence factors, other genes could be assigned to known biological functions: metabolism, regulation, motility and resistance to stress. This method could be easily implemented to other plant pathogenic bacteria and other plants.

"Stealth and brute force behaviour of *Pectobacterium atrosepticum* inside the plant"

Vladimir Gorshkov[1,2], Rim Gubaev[1], Amina Daminova[1], Olga Petrova[1], Marina Ageeva[1], Natalia Gogoleva[1,2], Olga Parfirova[1,2], Bakhtiyar Islamov[1], Polina Mikshina[1], Yuri Gogolev[1,2]

[1]Kazan Institute of Biochemistry and Biophysics, Kazan Science Centre, Russian Academy of Sciences, Kazan, Russia
[2]Kazan Federal University, Kazan, Russia

*e-mail: gvy84@mail.ru, vladimir.gorshkov@kibb.knc.ru
*Tel: +7 (843) 292-72-22

Pectobacteria known to utilize brute force for plant colonization are also considered to behave as stealth pathogens. We have shown that stealth behaviour of pectobacteria is associated with colonization of the primary xylem vessels where bacteria form specific multicellular structures – bacterial emboli. These structures represent products of plant-microbe integration, since their formation and maturation requires the action and the metabolites of both macro- and microorganisms. In a form of bacterial emboli, pectobacteria implement symptomless systemic colonization of the plant. The NGS analysis have revealed the features that distinguish stealth and brute force behaviour of pectobacteria *in planta* and allowed identification of a switcher (coronafacic acid, cfa) that determined a transition from ”amenable” to “devastating” mode of action. *cfa* mutant was able to systemically colonize xylem vessels but was impaired in colonizing core parenchyma and causing soft rots.

The study was supported by RSF (No 15-14-10022).

"The influence of two quorum sensing systems on the phenotypic features of *Dickeya solani* exhibiting different virulence levels"

Marta Potrykus[1], Nicole Hugouvieux-Cotte-Pattat[2] and Ewa Łojkowska

[1]Department of Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Abrahama 58, 80-307 Gdansk, Poland
[2]UMR5240 Microbiologie Adaptation et Pathogénie, Univ Lyon, CNRS, Univ Claude Bernard Lyon 1, INSA Lyon, F-69621 Villeurbanne, France

Dickeya solani strains of distinct origin show genetic homogeneity but significantly differ in their virulence. *Dickeya* species possess two quorum sensing (QS) mechanisms: the classic one based on N-acyl-homoserine lactone (AHL) signals and a specific one depending on the production and perception of an unknown molecule (VFM). To study the influence of these QS, mutants were constructed by inactivating genes coding for each system. Double mutants were obtained by simultaneous inactivation of genes coding for both QS. The inactivation of both QS in *D. solani* did not reveal any additive effect on the tested features. The inactivation of vfm genes has generally preponderant effect on that of the exp genes. Thus, VFM- and AHL-QS do not work in synergy to modulate the production of diverse virulence factors and the ability to macerate plant tissue.

Acknowledgements

The work was supported by Polish National Science Center with the project UMO-2014/14/M/NZ8/00501 and UMO-2014/13/N/NZ9/01081.

"Interkingdom signaling: interference of plant derived small molecules with bacterial communication and virulence"

Iris Yedidia
Plant Science Institute, Agricultural Research Organization, Volcani Center, Bet Dagan, Israel

Plants produce diverse array of low molecular-mass compounds, with more than 8,000 phenolics. Here, we provide an example for interference of plant small molecules (phenolics) with bacterial virulence, via inhibition of the quorum sensing (QS) machinery of the soft rot bacterium *P. carotovorum*. Interestingly, biofilm formation and exoenzymes activity were significantly impaired, at compound concentrations that did not affect bacterial cell growth or membrane integrity. Since biofilm production and exoenzymes are virulence determinants known to be under the strict control of QS, the effect of specific molecules on QS was studied. Common volatile and soluble phenolic compounds were tested for their influence on the expression of central QS system and QS controlled genes. The N-acyl-homoserine lactone (AHL) reporter strains (CV026 and pSB401) demonstrated a prominent reduction in the level of QS signal molecules accumulation, following exposure to the compounds. Moreover, infection capabilities were strongly impaired on potato, cabbage and calla-lily; but fully recovered upon external application of AHL. *Pectobacterium* central QS proteins ExpI/ExpR were used as targets for salycilic acid and carvacrol (plant defense molecules) using SCHRODINGER® molecular docking suit, to show possible interactions. Finally, by using isothermal calorimetry (ITC), SA and CAR were directly bound to cloned and purified ExpI, by this, experimentally supporting the computational docking results. True binding of plant derived phenolics to bacterial QS synthase protein as a target was demonstrated.

"Transmission of blackleg symptoms and latent infection from plant to plant under greenhouse conditions"

Patrice de Werra, Nicolas Linder, Alan Storelli and Andreas Keiser
School of Agricultural, Forest and Food Sciences (HAFL), Bern University of Applied Sciences, Switzerland

In Switzerland, *Pectobacterium carotovorum* subsp. *brasiliense* (*Pcbr*) is widely found as latent infection in imported and national seed lots but does not express systematically visible blackleg symptoms in the field. In contrast, the less frequent *Dickeya* sp. (*Dsp*) seems to be, once expressed in the field, more aggressive. Based on these observations, we developed an artificial greenhouse assay to study the transmission of

blackleg (symptoms and latent infection) from plant to plant on a plain with a downward slope of 8%. Three seed potatoes of two different cultivars were inoculated with either *Dsp* or *Pcbr* and planted on the upper part of the plain. Twelve non-inoculated potatoes were planted downstream of inoculated tubers. A drip irrigation system provided water at the top of the plain, allowing the water to flow downwards to the non-inoculated plants. During vegetation, the potato plants were checked for blackleg symptoms. After tuber formation, haulm killing was performed and all daughter tubers harvested and analysed for latent infection. The results of two distinct assays are presented here.

Additional Information

Bus/tram/taxi from airport to hotel

Both the Airlink 100 bus and the tram from the airport pass the hotel. However, there are currently road works closing Haymarket Terrace eastbound (ie from the airport), with the bus turning left and stopping on Magdala Crescent instead and rejoining Clifton Terrace via Grosvenor Street. You can get off before the hotel at Magdala Crescent or after at West Maitland Street. - see map overleaf.

Airlink 100 (Stop D)

This express bus service runs from the airport to Waverley Bridge (near Princes Street and the main rail and bus stations).

Buy tickets in advance online, from Visitscotland by UK Arrivals, at the bus stop, or from the driver

- Frequency: Every 10 minutes.
- Fare: Single £4.50, return £7.50

Tram (outside airport east entrance)

Tram fares between the Airport and City centre are:

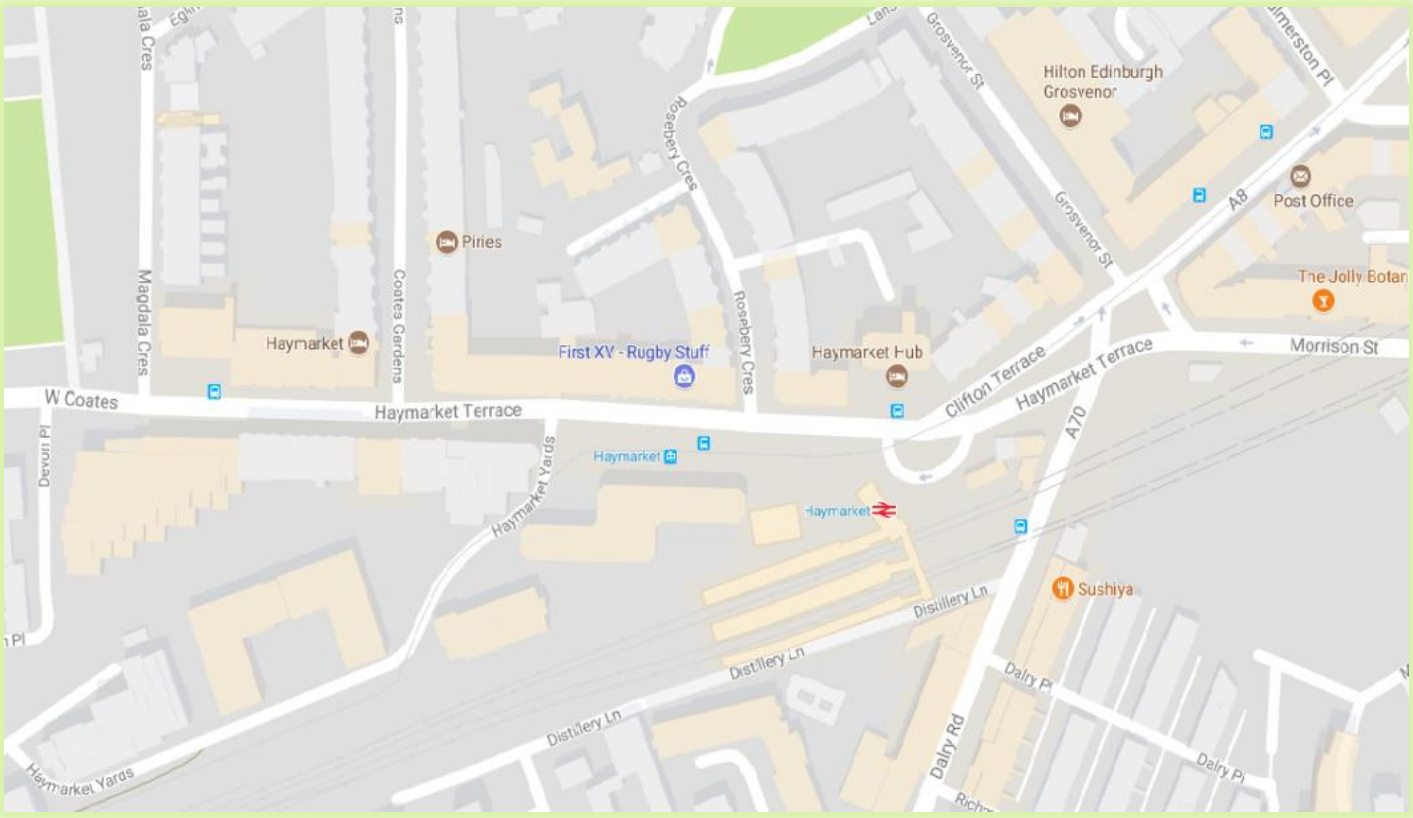
- Adult single £5.50
- Adult return £8.50

Buy your ticket before you board. Tickets can be purchased from ticket vending machines at the airport tram station. Ticket vending machines accept most debit and credit cards (£3 minimum spend) as well as 5p, 10p, 20p, 50p, £1 and £2 coins. No change is given. Edinburgh Trams recommends purchasing return tickets to make your journeys simpler. Return tickets are open ended meaning the return portion can be used when needed.

Taxis

Taxis are usually plentiful and are located on the ground floor of the multi-storey car park opposite the main terminal building – see map for location of tram & taxi rank stop.

For further information go to http://www.edinburghairport.com/transport-links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Distillery Ln
Dairy Rd
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A70
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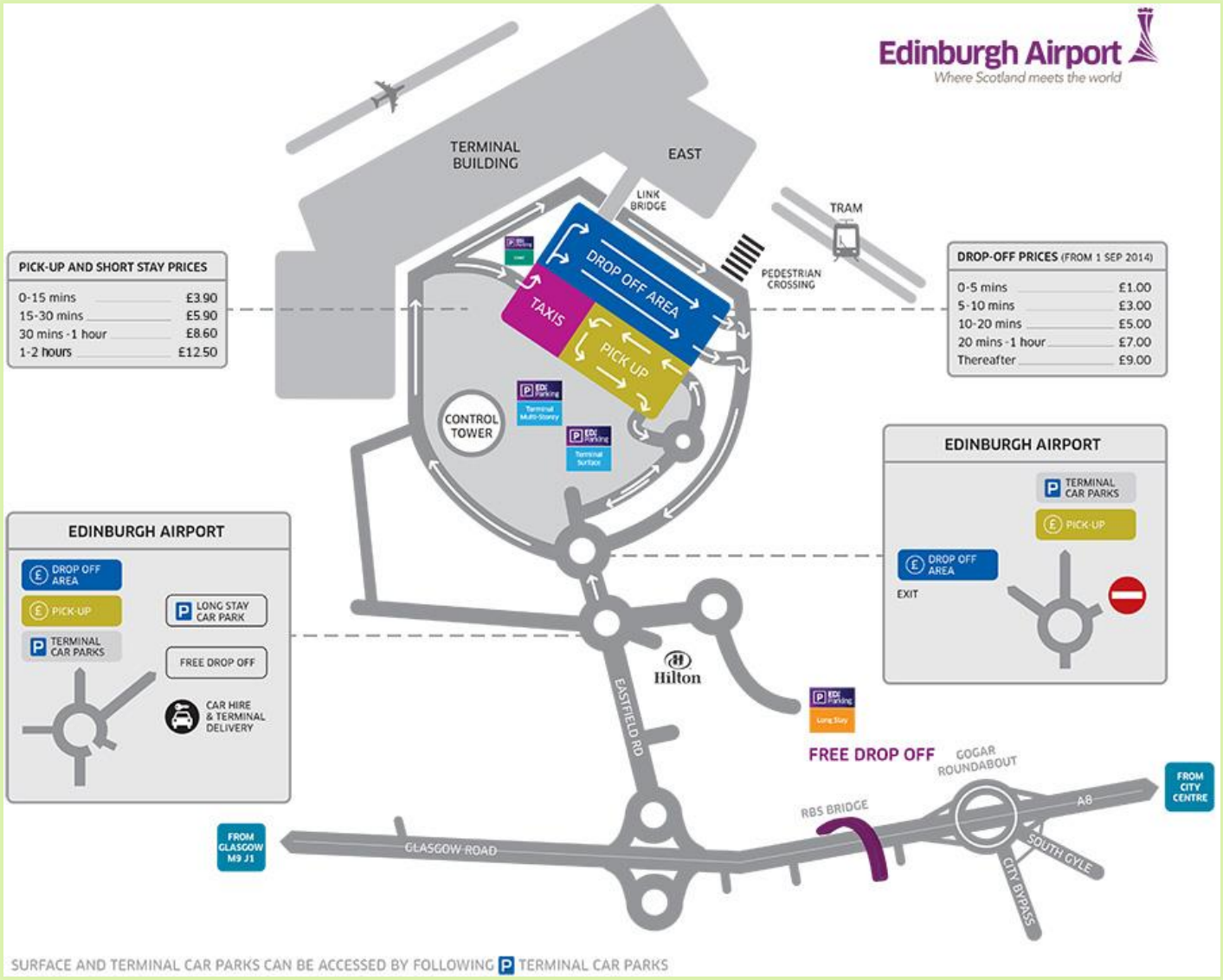
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DROP OFF AREA
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CONTROL TOWER
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15-30 mins £5.90
30 mins -1 hour £8.60
1-2 hours £12.50
DROP-OFF PRICES (FROM 1 SEP 2014)
0-5 mins £1.00
5-10 mins £3.00
10-20 mins £5.00
20 mins -1 hour £7.00
Thereafter £9.00
EDINBURGH AIRPORT
DROP OFF AREA
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LONG STAY CAR PARK
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CAR HIRE & TERMINAL DELIVERY
EDINBURGH AIRPORT
TERMINAL CAR PARKS
PICK-UP
DROP OFF AREA
EXIT
Hilton
EASTFIELD RD
FREE DROP OFF
GOGAR ROUNDABOUT
RBS BRIDGE
A8
FROM CITY CENTRE
FROM GLASGOW M9 J1
GLASGOW ROAD
SOUTH GYLE
CITY BYPASS
SURFACE AND TERMINAL CAR PARKS CAN BE ACCESSED BY FOLLOWING TERMINAL CAR PARKS

Tour

If you would like to go on the walking tour, Greig will meet you in the Haymarket Hub Hotel foyer at 8:50am, and we will get a bus to Edinburgh's Old Town.

(LRT Bus No. 31. Cost £1.60 for single journey, no change given on bus. Greig will bring coins in case people need change)

Tour details

The true history of the Blair Street Underground Vaults is terrifying enough – so abandon thoughts of manufactured frights and make-believe. The skill is in the storytelling: the vaults witnessed the deeds of mischief-makers and murderers, vagrants, and torturers. It's one of Edinburgh's most haunted sites, where restless spirits still tread.

First, you'll follow your cloaked Mercat guide through the shadowy closes of Edinburgh's Old Town. These are dark streets untouched by daylight, filled with a strange silence, their damp walls carrying only the faintest echo of the crowds on the high street you've left behind.

With the scene set, you'll descend into the depths of the city – and listen in horror to its dark and sinister side. Torture. Murder. Hangings. From body snatchers to the real-life Jekyll and Hyde, you'll be gripped by the true tales that lie hidden beneath Edinburgh's streets.

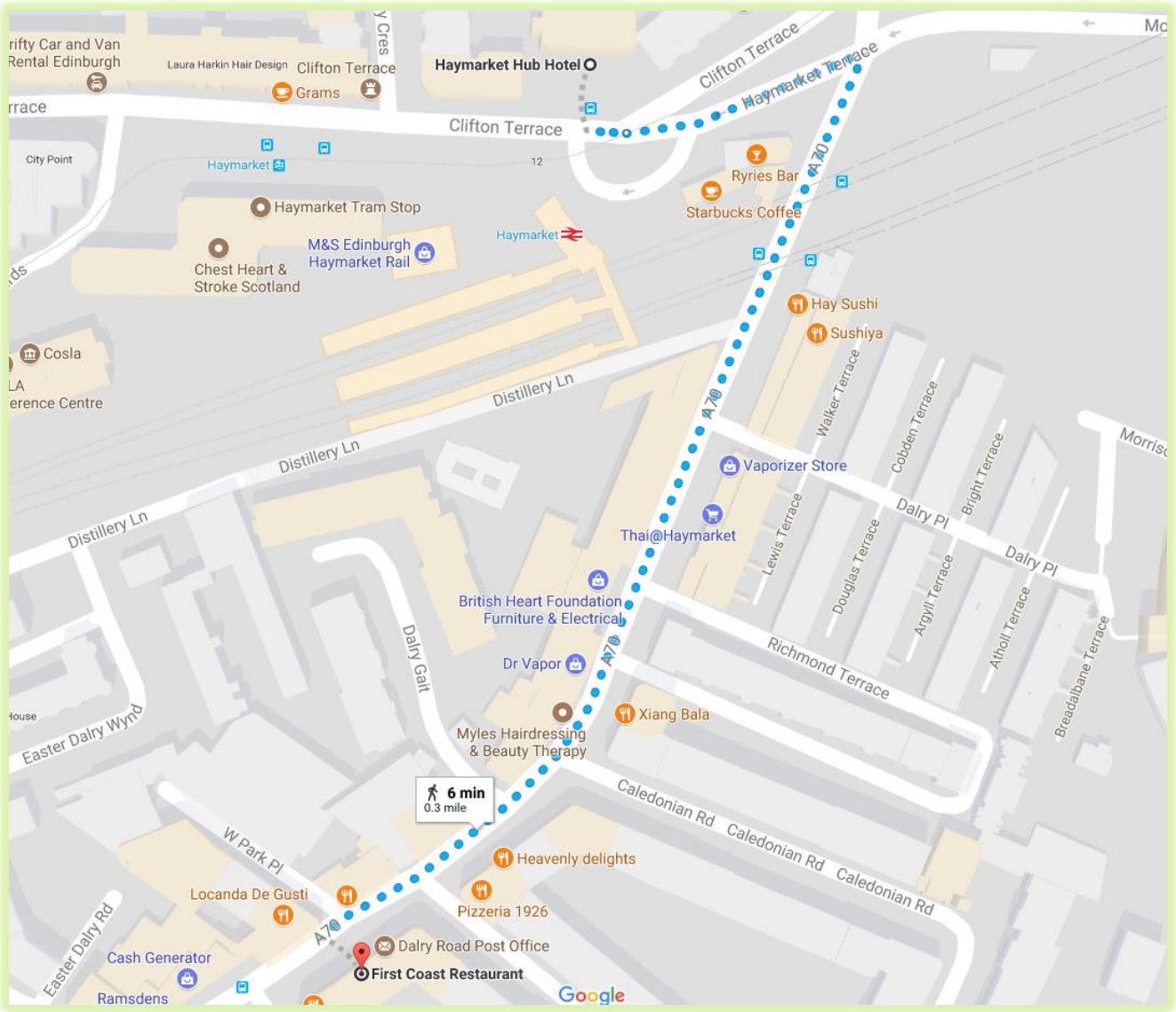
Watch out for a cold breath on the back of the neck, a whisper, a flicker – then silence. It's all the more chilling for being absolutely real: there are no tricks or jump scares on our ghost tours.

This tour is perfect for a peek into the city's ghostly past because things don't only go bump in the night. This tour may not be suitable for the faint of heart, or those who suffer from claustrophobia!
If you choose not to go on the tour, the bus will pick you up at 11:30am on the way to SASA.

Dinner

The conference dinner will be held in First Coast restaurant: just a short walk from Haymarket – see map below. Named after a tiny village in Wester Ross, First Coast is a quality neighbourhood bistro offering fresh, tasty homemade food in a relaxed environment.

https://www.first-coast.co.uk



Contact us

Any problems while in Edinburgh call

Greig: 0774 7788999
Gerry: 0782 5356028

List of Participants

Name	Email
Ivette Acuña	iacuna@inia.cl
Marie-Anne Barny	marie_anne.barny@agroparistech.fr
Amélie BEURY	amelie.beury@comitenord-sipre.fr
Alison Blackwell	ablackwell@apsbiocontrol.com
Doretta Boomsma	Doretta.Boomsma@hzpc.nl
Greig Cahill	Greig.Cahill@sasa.gsi.gov.uk
Jeremy Cigna	jeremy.cigna@comitenord-sipre.fr
Guy Condemine	guy.condemine@insa-lyon.fr
Triona Davey	Triona.Davey@sasa.gsi.gov.uk
Patrice de Werra	patrice.dewerra@bfh.ch
Yeshitila Degefu	yeshitila.degefu@luke.fi
Brice Dupuis	brice.dupuis@agroscope.admin.ch
Jakub Fikowicz Krosko	jakub.fikowicz-krosko@phdstud.ug.edu.pl jakub.fikowicz-krosko@biotech.ug.edu.pl
Vladimir Gorshkov	gvy84@mail.ru
Mónica Gutierrez	-
Tjaart Hofman	hofman@certiseurope.com
Sonia Humphris	Sonia.Humphris@hutton.ac.uk
Miriam Kooman	M.Kooman@nak.nl
Christophe Lacomme	Christophe.Lacomme@sasa.gsi.gov.uk
Yves le Hingrat	yves.lehingrat@fnpppt.fr
Ewa Łojkowska	ewa.lojkowska@biotech.ug.edu.pl
Rachel Mann	Rachel.Mann@ecodev.vic.gov.au
Minna Pirhonen	minna.pirhonen@helsinki.fi
Marta Potrykus	marta.potrykus@biotech.ug.edu.pl
Mirjam Prinz	Mirjam.Prinz@lfl.bayern.de
Leighton Pritchard	Leighton.Pritchard@hutton.ac.uk
Viviana Rivera-Varas	viviana.rivera@ndsu.edu
Åsa Rölin	Asa.Rolin@hushallningssallskapet.se
Emily Ross	Emily.Ross@sasa.gsi.gov.uk
Gerry Saddler	Gerry.Saddler@sasa.gsi.gov.uk
Camila Sandoval	-
Gary Secor	gary.secor@ndsu.edu
Ian Toth	Ian.Toth@hutton.ac.uk
Jacquie van der Waals	Jacquie.vanderWaals@up.ac.za
Jan van der Wolf	jan.vanderwolf@wur.nl
Frederique VAN GIJSEGEM	vangijse@agroparistech.fr
Robert Vreeburg	r.vreeburg@nak.nl
Lauren Watts	Lauren.Watts@hutton.ac.uk
Iris Yedidia	irisy@volcani.agri.gov.il

Euphresco
Network for phytosanitary research coordination and funding

Euphresco III
Dickeya/Pectobacterium Workshop

15 and 16 November 2018
NAK, Emmeloord, The Netherlands

Programme and Abstracts

Euphresco III
Dickeya/Pectobacterium Workshop

15 and 16 November 2018
NAK, Emmeloord, The Netherlands

Local organisers

- Jan van der Wolf, Wageningen UR
- Miriam Kooman, NAK
- Doretta Boomsma, HZPC B.V.

Coordinators Euphresco Dickeya/Pectobacterium project

- Maria Bergsma-Vlami, NVWA
- Jan van der Wolf, Wageningen UR

Coordinator Euphresco programme

- Baldissero Giovani (EPPO)

Sponsors

NAK

HZPC
Growing with our potatoes

Programme

Thursday 15 November (chair Miriam Kooman)

8.30 h Departure bus from Hotel Apollo, (Agoraweg 11, Lelystad) to the NAK (Randweg 14, Emmeloord)

9.00 h Welcome by Dr Eric Casteleijn (director NAK)

9.10 h Introduction workshop (Jan van der Wolf)

Surveys and ecology

9.25 h Leah Trsor. Characterization of *Pectobacterium carotovorum* subsp. *brasiliense* in Israel and seed treatments to control tuber soft rot

9.50 h Yeshitila Degefu. Extreme weather conditions and characterization of blackleg on potato during the 2018 cropping season in Finland

10.15 h Jianjun Hao. Tracking the bacteria associated with the outbreak of blackleg of potato in the Northeastern US

10.40 h Coffee/Tea break

11.15 h Marie Anne Barny. SRP in water environment, when, where and who is there?

11.40 h Robert Vreeburg. Virulence of different *Pectobacterium brasiliense* isolates tested at two different Dutch locations

12.15 h Vladimir Gorshkov. Non-conventional ways of pectobacteria adaptation to stress conditions

12.40 h Lunch

Genetic characterization of strains

13.30 h Nicole Hugovieux Cotte-Pattat. Abundance of *Dickeya* isolates in lakes from the French region La Dombes, including a new species, *D. lacustris*

13.55 h Minna Pirhonen. *Pectobacterium polaris* strains isolated in Finland

14.20 h Frédérique van Gijsegem. *Dickeya* diversity: Follow-up

15.05 h Coffee/Tea break

15.30 h Ewa Lojkowska. Genomic variability in the plant pathogenic bacterium *Pectobacterium parmentieri* deduced from *de novo* assembled complete genomes

15.55 h Jeremy Cigna. Contemporary pathogens: Description of *Pectobacterium punjabense sp. nov.* and sensitivity to biocontrol agents

16.20 h Malgorzata Waleron. Characterization of the two new species: *Pectobacterium peruviense* and *P. zantedeschiae* - can they pose a problem?

16.45 h Eef Jonkheer. Pangenome analysis and its application on Pectobacterium

17.10 h Valérie Hélias. Diversity and phylogeny of the *Pectobacterium* complex

17.35 h Jan van der Wolf. Metagenomics for detection and characterization of soft rot *Pectobacteriaceae* in air samples

18.30 h Departure for the workshop dinner (Urk)

Friday 16 November (chair Jan van der Wolf)

8.30 h Departure bus from Hotel Apollo, Lelystad to the NAK (Randweg 14, Emmeloord)

Plant pathogen interaction

9.00 h Iris Yedidia. Prospects on host specificity and differential pathogenicity of *Pectobacterium* spp.

Control

9.25 h Robert Czajkowski. Compatible mixtures of antagonistic bacteria developed specifically for biological control of Soft Rot *Pectobacteriaceae* (*Pectobacterium* spp. and *Dickeya* spp.) in potato (project PATBIOCON)

9.50 h Agata Motyka. Direct current atmospheric pressure glow discharge in possible agricultural applications

10.15 h Coffee/Tea

10.40 h Carina Eisfeldt. Survival of plant pathogenic bacteria in tile drainage water and the potential application of managed aquifer recharge in agriculture for safe reuse of irrigation water

11.05 h Ivette Acuna. Evaluation of the resistance to soft rot of commercial potato varieties in Chile

11.30 h Johannes Ransijn. Can complex systems approaches help to reduce disease incidence?

Diagnostics

11.55 Leighton Pritchard. Computational design of *Pectobacterium* diagnostics

12.20 Lunch

General discussion

- Trends in research on Pectobacterium/Dickeya

- Prospects on management of the disease

- Follow up Euphresco project and coordination of the follow up

- Next meeting

- Update "Erwinia book"

Tour de NAK

16.00 h Departure to Hotel Apollo

Saturday 17 November

10.00 – 13.00 h Sightseeing Amsterdam (see information at the end of the programme

Characterization of *Pectobacterium carotovorum* subsp. *brasiliense* in Israel and seed treatments to control tuber soft rot

Leah Tsror[1], S. Manulis-Sasson[2], S. Lebiush[1], O. Erlich[1], I. Galilov[1], L. Chalupowicz[2], M. Reuven[2], O. Dror[2], U. Zig[3]

[1]Department of Plant Pathology and weed Research, Agricultural Research Organization (ARO), [1]Gilat Research Centre, [2]The Volcani Center, [3]Yaham Enterprises Ltd., Israel.

Potato cultivation in Israel is carried out in spring season using seed tubers imported from Europe and in fall-winter with local seeds, produced during the spring under the inspection of Plant Protection and Inspection Services. Pectinolytic bacteria, primarily *Dickeya solani* (Ds), *Pectobacterium carotovorum* (Pc), *P. c. brasiliense* (Pcbr) and *P. parmentieri* (Pp), are the causal agents of pre-emergence seed rot, blackleg and wilt and tuber soft rot causing economic losses in both seasons. Disease symptoms vary with climate and development and expression of the disease in field may be particularly favored under warm-climate conditions which prevail in Israel. Latent infections with Ds and Pcbr in imported seed material directly affect the crop yields in both seasons. Pcbr strains obtained from European imported seeds and from potato plants and progeny tubers grown in Israel were characterized by Pulse Field Electrophoresis Gel (PFGE) and by sequencing the *gapA* gene. A major group of 40 strains (out of 60 tested strains) produced the same pattern with either *Avr*II or I-*Ceu*I. Their pathogenicity was evaluated by tuber maceration test. A considerable variation in maceration ability was observed within the tested strains related to the source of the plant material.

Seed tuber treatments were evaluated in reducing tuber rot during the fall-winter season in Israel, especially at early planting during September when temperatures are relatively high. The seed treatments included dry steam, oxolinic acid (Summit Agro International Ltd), mancozeb and MB5K (surface sterilizing agent, Adama Ltd) applied prior to planting. All treatments reduced seed decay or tuber rot, however, the results were inconsistent in field trials conducted in different years. The potential use of these treatments by farmers is considered.

Extreme weather conditions and characterization of blackleg on potato during the 2018 cropping season in Finland

Yeshitila Degefu

Natural Resources Institute Finland (LUKE)

Paavo Havaksen tie 3

FI-90014 University of Oulu, Finland

E-mail: yeshitila.degefu@luke.fi

According to the report of the Finnish Meteorological Institute, the summer from May to August 2018 in Finland was warmer than ever in the recorded history. July was the warmest month ever recorded in the history of the institute. There were a total of 27 hot days, days when temperatures rose over 25 ^{0}C, in July alone. The summer was also characterized by exceptionally dry periods in most parts of the country. Extreme in both aspects. Therefore, the impact of these extreme weather conditions during the cropping season on the prevalence of blackleg on potato and the species of *Dickeya* and *Pectobacterium* predominant in the observed disease complex in the season is of particular interest. Diseases survey was conducted in the major seed potato growing areas in North Finland and ware potato production localities in the South Ostrobothnia near central Finland were carried out. Results from the characterization of observed outbreaks of blackleg from those areas and data obtained from routine lab tests for latent infection of seed tubers will be discussed.

Tracking the bacteria associated with the outbreak of blackleg of potato in the Northeastern US

Hao JJ[1*] Ge T[1] Marangoni N[1] Jiang H[1] Johnson SB[2] Larkin RP[3]

[1]*School of Food and Agriculture,*

[2]*Cooperative Extension*

[3]*USDA-ARS, New England Plant, Soil, and Water Lab University of Maine, Orono, ME 04469, USA*

**Email jianjun.hao1@maine.edu*

An outbreak of blackleg of potato, predominantly caused by *Dickeya dianthicola,* occurred in the Eastern states of the US beginning in 2015. Symptomatic potato plants were collected from Maine and the states where Maine seed tubers were planted and used to isolate blackleg pathogens. By analyzing 125 isolates of pectolytic bacteria with multi-locus sequence analysis, Maine isolates were less diverse than those from the other states, indicating that outbreaks in other states may not have only originated from Maine seed tubers. Isolates collected from soil, non-potato plants, and surface water were analyzed but were not associated with the disease epidemic. It is probable that the outbreak resulted from multiple factors, which will be discussed.

SRP in water environment, when, where and who is there?

J Pédron[1], Berge O[2,] ,Van Gisjegem F[1], and Barny Ma[1]

[1] *Institute of Ecology and Environmental Science-Paris*

University Pierre and Marie Curie - 4 place Jussieu - 75252 PARIS cedex 05

[2]*INRA,Plant Pathology Research Unit, 84143 Montfavet, France*

Soft Rot *Pectobacteriaceae* (SRP) diversity is well characterized on disease crop species in agricultural context. In contrast, their abundance, repartition and diversity outside the plant context remain largely unexplored. Early studies showed that SRP could be recovered from various non-host environments, such as water, soil or air. However, the taxonomic status of most of the isolated strains in these early works was not clearly assigned since most species were described later on. Therefore, is it still unclear which particular species could be recovered from various non-host environments. An effort to analyse the situation is particularly important with water since water could serve for irrigation purposes. We analysed the current situation at the scale of a whole catchment area, from pristine alpin water to irrigation water in arable land. Two years survey at the scale of the Durance catchment area was performed in 2016 and 2017. Sampling was performed four times a year in winter, spring, summer and fall on 21 sites on the main river and its tributaries. Plating water on CVP allowed us to survey the SPR concentration at each sites during the two consecutive years. Isolation of nearly 800 SPR and sequencing of the *gap*A housekeeping gene allowed us to characterize the isolated strains. The result of these two years survey will be presented.

Virulence of different *P. c.* subsp. *brasiliense* isolates tested at two different Dutch locations

[1]Robert Vreeburg, [1]Eisse de Haan, [1]Mathijs Nas[1] (and others), [2]Martzen ten Klooster, [2]Doretta Boomsma, [3]Jan van der Wolf, [4]Johan van Vaerenbergh, [5]Jack Gros

[1]NAK, Randweg 16, Emmeloord

[2] HZPC B.V., Edisonweg 5, 8501 XG Joure

[3] WUR, P.O. Box 16, 6700 AA Wageningen

[4] ILVO, Burgemeester Van Gansberghelaan 92 bus 1, 9820 Merelbeke

[5] Agrico Research, Burchtweg 17, Bant

Email: r.vreeburg@nak.nl

A Belgian-Dutch consortium of NAK, HZPC, WUR, ILVO and Agrico has tested different *Pectobacterium carotovorum* subsp. *brasiliense* (Pcb) isolates at two test sites in The Netherlands. The panel of Pcb isolates contained isolates from symptomatic potato plants, latently infected tubers, one isolate from insects and one from another latently infected crop. Tubers of the varieties Kondor and Spunta (class PB3) were vacuum inoculated with the different isolates both at the NAK and at HZPC, sharing the same potato tuber batch and isolates, but performing the experiment independently. Inoculated tubers were planted in four randomised blocks, with a total of 96 tubers per isolate. Wilting symptoms were scored weekly and accumulated symptom development was used as measure for virulence. Different isolates showed different virulence, with a similar picture at both sites. The virulence in Kondor was mirrored by the virulence in Spunta at the NAK site, but no additional symptoms above mock level were observed in Spunta at the HZPC site.

Virulence of a subset of isolates was tested by dipping inoculation, resulting in lower disease symptoms, but the isolates showed a similar ranking of the virulence as with vacuum inoculation. The virulence of the same subset was also tested on different varieties with minitubers, resulting in similar virulence results on the different potato varieties.

Some isolates that originate from symptomatic plants, and many isolates from latent tubers, were not or weakly virulent in the field experiments. One explanation for this is that the reduced-virulence of the isolates is an artefact of the isolation procedure. This hypothesis still needs to be tested in a new field experiment.

Non-conventional ways of pectobacteria adaptation to stress conditions

Vladimir Gorshkov[1,2*], Olga Petrova[1], Amina Daminova[1], Marina Ageeva[1], Natalia Gogoleva[1,2], Bakhtiyar Islamov[1], Vladimir Vorob`ev[1,2], Yuri Gogolev[1,2]

[1]Kazan Institute of Biochemistry and Biophysics, Federal Research Center "Kazan Scientific Center of RAS"

[2]Kazan Federal University, Kazan, Russia

**Email gvy84@mail.ru, vladimir.gorshkov@kibb.knc.ru*

One of the major gaps in the understanding of soft rot pathogen biology is a limited information about the way these bacteria survive in various stressful ecological niches (e.g. soil, waste water, air, plant remnants) when being out of the host plant. The conventional concept states that in order to adapt, the non-spore-forming bacteria stop proliferation to divert resources to effectively resist stress factors by forming particular specialized cell morphotype or phenotype (e.g. viable but non-culturable cells, cell wall deficient forms, persisters, etc.). An alternative sigma factor RpoS as well as quorum sensing system (QS) are considered to be crucial molecular players in coordination of bacterial stress response. QS regulation of stress response defines that the phenomenon of bacterial adaptation is realized not at a cell but at the population level and requires cell-to-cell communication taking place only at high population density. Besides, it is believed that to effectively survive under stress effect, bacteria should be "pre-prepared" by moderate stress that stops fast proliferation and induces accumulation of storage compounds; in other words, bacteria of the stationary growth phase adapt much more effectively than those of log growth phase.

However, the bacteria may encounter stress factors either during active proliferation, or being at a low population density. Therefore, we proposed the existence of non-conventional ways of bacterial adaptation. We have revealed and described the phenomenon of adaptive proliferation, when stress response is associated not with the growth arrest but with a significant increase in population density even in the absence of an exogenous nutritional substrate. Adaptive proliferation occurs only in those cases when the population density is too low to implement cell-to-cell communication, and is evidently supported by endogenous cell resources. The increase of population density in this case proceeds until the cell titer reaches the quorum level and the bacteria acquire cross-protection. Additionally, we described the log phase cell adaptation compared to that of the stationary phase cells. Besides, although adaptation is considered to be a non-specific response, we have found that different stressors induce alternative regulatory cascades and that pectobacteria adaptation to some of them does not require functional *rpoS* gene. This study was partially supported by a grant MK-2191.2017.4 and Russian Science Foundation (project No. 17-14-01363).

Abundance of *Dickeya* isolates in lakes from the French region La Dombes, including a new species, *D. lacustris*

Hugouvieux-Cotte-Pattat N[1]*, Jacot-des-Combes C[2], Briolay J[2]

[1]*UMR5240 Microbiologie Adaptation and Pathogenesis,*

[2]*Plateforme DTAMB,*

University Lyon 1, CNRS, INSA, F-69621Villeurbanne, France

**Email* Nicole.Cotte-Pattat@insa-lyon.fr

To better understand the natural diversity of *Dickeya*, a survey was performed in small lakes surrounded by wetlands in the French region of La Dombes. Several *Dickeya* isolates were obtained from lakes protected from direct agricultural inputs. Sequencing of the gene *gapA* revealed the presence of three already characterized species, *D. zeae*, *D. chrysanthemi* and *D. aquatica*. Other isolates belong to a phylogenetic group separated from the known *Dickeya* species. Genomic analysis supported the delineation of a new species for which the name *Dickeya lacustris* was proposed. The closest species is *D. aquatica,* previously isolated from rivers, suggesting that these two species have a common ancestor adapted to a water environment.

Pectobacterium polaris strains isolated in Finland

Miia Pasanen[1], Thomas Schott[2], Yeshitila Degefu[3], Virpi Ahola[4], Leighton Pritchard[5] and *Minna Pirhonen[1]*

[1]Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland

[2]Herne Genomic, Neustadt, Germany

[3]Luke Natural Resources Institute Finland, Oulu, Finland

[4]Department of Biosciences, University of Helsinki, Helsinki, Finland

[5]Information and Computational Sciences, The James Hutton Institute, Dundee, Scotland, United Kingdom

Pectobacterium polaris is a newly identified pectinolytic and highly virulent plant pathogen isolated in Norway from potato samples. Five *Pectobacterium* isolates identified from potato stems in Finland were previously characterised as *Pectobacterium carotovorum* subsp. carotovorum but were not recognized in PCR tests that detect various *Pectobacterium* species. Average Nucleotide Identity (ANI) revealed that the representatives of the Finnish isolates, s0416 and s0421, shared 96% identity with *Pectobacterium polaris* strains NIBIO 1006^{T} and NIBIO 1392, and over 99% identity with *P. polaris* strain NCPPB 3395 and isolate YC T1 still included into *P. carotovorum* in NCBI. However, based on MLSA and GGDC analyses and genome comparisons, the Finnish isolates and NCPPB 3395 were found to be differentiable from the *P. polaris* isolates NIBIO 1006^{T} and NIBIO 1392, possibly representing a separate subspecies. The implication of these results for the taxonomy of the Finnish isolates is discussed.

Dickeya diversity: Follow-up

Frédérique Van Gijsegem

iEES Paris (Institute of Ecology and Environmental Sciences), INRA-Sorbonne Universités UPMC Paris 06, UMR 1392, F-75005, Paris, France

**Email vangijse@agroparistech.fr*

Eight species have been described in the *Dickeya* genus sofar: *D. dadantii, D. dianthicola, D. zeae, D. chrysanthemi, D. paradisiaca, D. solani, D. aquatica* and *D. fanghzondai*. This was however based mostly on strains isolated from diseased plants. To analyse the *Dickeya* diversity in another environment, we performed an extensive survey of the population of pectinolytic bacteria collected in a water canal near Avignon in the South of France. Most *Dickeya* species were found as well as some isolates belonging to a yet unidentified genomic clade including a strain isolate from a lake in Asia. We also isolated different *D. solani* strains. *D. solani* was mainly isolated from potato and ornamentals and appeared to be clonal, differing only by a few dozen SNPs/InDels except for one divergent strain. A recent reclassification of all former *Erwinia chrysanthemi* strains present in the CFBP collection into the different *Dickeya* species allowed the identification of a new *D. solani* strain that was isolated from a previously non-identified host – tomato - in Guadeloupe. A similar analysis of the Swiss collection of former *E. chrysanthemi* strains isolated from potato since the 1980s and our water survey led to the isolation of other *D. solani* strains presenting different levels of diversity. This intra-specific diversity will be discussed.

Genomic variability in the plant pathogenic bacterium *Pectobacterium parmentieri* deduced from *de novo* assembled complete genomes

Ewa Lojkowska[1], Sabina Zoledowska[1], Agata Motyka[1], Wojciech Sledz[1], Alessio Mengoni[2]

[1]*Department of Biotechnology, Intercollegiate Faculty of Biotechnology UG and MUG, Poland.*

[2]*Department of Biology, University of Florence, Sesto Fiorentino, Florence, Italy*

Pectobacterium parmentieri is a causative agent of diseases in economically important crops (*e.g.* potato) in a wide range of different environmental conditions, encountered in Europe, North America, Africa, and New Zealand. Severe disease symptoms result from the activity of *P. parmentieri* virulence factors, such as plant cell wall degrading enzymes. Interestingly, we observe significant phenotypic differences among *P. parmentieri* isolates regarding the abilities to macerate plant tissue and activities of virulence factors. The pan-genome study was performed on 15 genomes:12 *de novo* assembled and three reference strains: *P. parmentieri* CFBP 8475^{T}, *P. parmentieri* SCC3193, *P. parmentieri* WPP163. The pan-genome includes 3 706 core genes and 3 315 dispensable genes; within them 1 468 accessory genes, and 1 847 unique genes. The presence of well-known genes encoding virulence factors was observed in the core genome fraction, but some of them were located in the dispensable genome. A significant fraction of horizontally transferred genes, virulence-related gene duplications, as well as different CRISPR arrays were found, which can explain the observed phenotypic differences. We can hypothesize that a large number of the genes in the dispensable genome and significant genomic variation among *P. parmentieri* strains could be the basis of its widespread diffusion and adaptation to different environments.

Contemporary pathogens: Description of *Pectobacterium punjabense* sp. nov. and sensitivity to biocontrol agents

Cigna Jérémy*[1-2], Robic Kévin[1-2], Dewaegeneire Pauline[1], Laurent Angélique[1], Gobert Virginie[1], Le Hingrat Yves[1], Beury-Cirou Amélie[1], Hélias Valérie[1], and Faure Denis[2].

[1] *National Federation of Seed Potato Growers (FN3PT-RD3PT), 75008 Paris, France*

[2] *CNRS-I2BC, ANR-15-CE21-0003, 1 avenue de la terrasse, 91198 Gif-sur-Yvette, France*

*Email: jeremy.cigna@fnpppt.fr

The characterization of isolates from the *Pectobacterium* genus, originating from blackleg symptoms in Pakistani potato fields have conducted to the recent description of a new species called *P. punjabense* in reference to the area where it was isolated. Here we present the MLSA studies, DNA-DNA hybridization and ANI values that clearly revealed the isolate SS95^T as a member of the new *P.punjabense* species. In complement, an *in-silico* analysis allowed us to identify specific genomic region present in *P. punjabense* and absent in the nearest *P. parmentieri* and *P. wasabiae* species. Phenotypic assay using various carbon sources indicates the same features for *P. punjabense* and *P. parmentieri* type strains. Maceration tuber assay with *P. punjabense* SS95^T was compared to others Pakistani isolates. Results revealed a level of aggressiveness equivalent to the *P. parmentieri* isolate and lower than the *D. dianthicola* and *P. atrosepticum* isolates. Finally, *P. punjabense* strains were occasionally identified in our collection and elsewhere in Europe, showing that this species is not restricted to Pakistan. Results will be discussed.

At the same time, biocontrol experiments were conducted in order to control the blackleg disease. One of the objectives is to study the sensitivity of contemporary pathogens regarding three biocontrol agents previously selected. In this context, 600 pectinolytic bacteria were isolated since 2013 from blackleg symptoms in French potato fields. After identification of all the isolates with "gapA phylogeny", a sample group of 41 isolates was selected, including one new *P. punjabense* isolate. Finally, their sensitivity regarding the biocontrol agents was studied. Results of antibiosis test showed a greater inhibition zone with one of the biocontrol agents compared to the others, whatever the pathogen isolate tested.

Characterization of the two new species: *P. peruviense* and *P. zantedeschiae* - can they pose a problem?

Waleron M[1], Misztak A[1] Waleron MM[1], Franczuk M[2], Jonca J[2], Wielgomas B[3], Mikiciński A[4], Popović T[5] and Waleron K[2]

[1] *Department of Biotechnology, University of Gdansk, Poland*

[2] *Department of Pharmaceutical Microbiology, Medical University of Gdansk, Poland*

[3] *Department of Toxicology, Medical University of Gdansk, Poland*

[4] *Research Institute of Horticulture, Skierniewice, Poland*

[5] *Institute for Plant Protection and Environment, Belgrade, Serbia;*

* *e-mail: malgorzata.waleron@biotech.ug.edu.pl*

The genus *Pectobacterium* was proposed for the first time by Waldee in 1945. Since then, the taxonomy of this genus underwent extensive remodelling. The appearance of the new and more affordable sequencing technologies resulted in the progressive increase in data pool allowing for better insight into variety within this genus. Studies based on the genomic data revealed the need for reclassification of several previously misassigned strains. As a result of the new species have been described, among them *Pectobacterium peruviense* and *Pectobacterium zantedeschiae.*

The new species *P. peruviense* gather strains isolated from potatoes tubers cultivated in the high altitude in Peru that can infect and efficiently macerate tissue in temp. range 12 – 28 °C. What's more, the bacteria belonging to this species are not only resistant to low temperature, but also to high salinity. Recently new *P. peruviense* strains were isolated from alpine rivers in France. The strains of *P. zantedeschiae*, which were isolated from Calla lily, are highly virulent and can infect both monocotyledonous and dicotyledonous plants. Strains from this species tolerate a wide range of salinity, pH, and water availability and also they show resistance to some antibiotics, isothiocyanate and bile salts.

Financed: NCN2015/17/B/NZ9/01730

Pangenome analysis and its application on Pectobacterium

Eef Jonkheer[1], Sandra Smit[1], Dick de Ridder[1], Balázs Brankovics[2], Ilse Houwers[2], Jan van der Wolf[2], Peter Bonants[2], Robert Vreeburg[3],Theo van der Lee[2]

1. Bioinformatics group, Wageningen University, Droevendaalsesteeg 1, 6708PB Wageningen, The Netherlands

2. Biointeractions & Plant Health, Wageningen Plant Research, Droevendaalsesteeg 1, 6708PB Wageningen, The Netherlands

3. Nederlandse Algemene Keuringsdienst, Randweg 14, 8304 AS Emmeloord

E-mail: theo.vanderlee@wur.nl

Next-Generation Sequencing (NGS) results in many, sometimes hundreds or thousands, of high-quality genome assemblies for a single species. Current reference based genome analysis pipelines cannot deal with such numbers effectively. To manage, store and exploit these data we need new strategies. Previous studies have shown that in a single species we can identify the core genome (present in all), the accessory part (present in some) and unique parts, therefore a single reference genome is simply inadequate to capture the genotypic diversity. To analyse multiple genomes simultaneously we developed PanTools (Sheikhizadeh et al,2016), an innovative platform for pangenomic analysis that is scalable and computational efficient. PanTools uses a localized de Bruijn graph as a pangenome representation and stores the data in a Neo4j graph database. This graph not only stores the sequence data, but by including annotations we can easily traverse the graph and interrogate the data to address biological questions. Within PanTools fundamental but complicated questions such as the identification of orthologous genes can be performed in a fraction of the time compared to popular methods such as Orthofinder. In addition PanTools allows linking metadata associated with the genomes to identify communalities between genomes that share a phenotype or other feature.

We will present some first results of our approach applying PanTools on the analysis of bacterial genome content and organization. We constructed a pangenome of 40 *Pectobacterium brasiliensis* of both in-house sequencing data as genomes from NCBI. Within this pangenome we performed various analyses such as calculation of ANI scores, clustering of proteins and estimation of the core, accessory and unique genome. In the 40 genomes we can distinguished at least three different clusters. We found 200 orthologous groups of genes specific to one of these clusters. The genes in these groups typically are physically linked in the genome. Using functional annotation tools we were able to identity candidates genes that could be important for the infection.

This project is financed by the PPS innovatieve diagnostiek. With partners NAK Emeloord, Naktuinbouw and BKD

Diversity and phylogeny of the *Pectobacterium* complex

Khaoula Chawki[1,2], Angélique Laurent[1,2], Géraldine Taghouti[3] , Emma Caullireau, Marion Fischer-le Saux[3], Yves Le Hingrat[1], Didier Andrivon[2] , Perrine Portier[3]**, Valérie Hélias**[1,2]

[1] FN3PT/RD3PT, French Federation of Seed Potato Growers, 43-45 rue de Naples, F-75008 Paris, France

[2] INRA, UMR1349 IGEPP, F-35653 Le Rheu Cedex, France

[3] CIRM-CFBP, IRHS, INRA, AGROCAMPUS-Ouest, Université d'Angers, SFR 4207 QUASAV, 42 rue Georges Morel, 49071 Beaucouzé cedex, France

Keywords : *taxonomy*, soft rot, MLSA, blackleg, certification

Pectobacterium contains to this day 11 official species after recent new assignments. Field surveys conducted annually in France since 2003, coupled with diversity studies of the resulting strain collections, showed that blackleg symptoms are associated with a much larger range of species than the one (*P. atrosepticum*) historically almost exclusively held responsible for these symptoms in Europe. We thus aimed at analyzing more deeply the diversity and the phylogeny of *Pectobacterium,* particularly strains isolated from field symptoms on potato, and at evaluating changes within the *P. carotovorum (P.c.)* group during the last decades. Refining the taxonomy of the *Pectobacterium* groups was needed to develop specific detection tools, useful for testing and certifying seed lots. A MLSA was performed using *recA*, *dnaX* and *leuS* genes on 384 strains from the FN3PT and the CIRM-CFBP collections; 53 *Pectobacterium* sequences from databases were used as supplementary data. The already described species including the recently described *P. polaris* and the subspecies *P. c. odoriferum*, *P. c. brasiliense*, *P. c. actinidiae* were clearly defined by the MLSA results. In contrast, strains from *P. carotovorum* subsp. *carotovorum* showed a high genetic diversity in accordance with our earlier sequencing work on *PelY*. The results showed that *P. carotovorum* subsp. *carotovorum* strains were distributed in several groups, all not being previously described. Among them, one corresponded to the "putative *P. maceratum"*. Additionally, some orphan strains were identified, that were not allocated to any specific cluster, except one, belonging to the *P. punjabense* species (see Cigna *et al*.).

Metagenomics for detection and characterization of soft rot *Pectobacteriaceae* in air samples

Van der Wolf, J.M.[1*], Dullemans, A.[1], Krijger, M.[1], Putra, G.[1], Warris, S[1]. & Fokkema, J.[2]

[1]*Wageningen University & Research, NL*

[2]*NAO (NAO; Dutch Organisation of Potato Merchants), NL*

**Email jan.vanderwolf@wur.nl*

In the past, it has been shown that soft rot *Pectobacteriaceae* can be spread from infected potato fields via aerosols, generated due to splashing water or during (mechanical) haulm destruction. Analysis of sampled aerosols can be important in studies on transmission of the pathogen but may also be efficient in the diagnosis of fields. In our studies, 24 air samples were collected with a Coriolis air sampler in different seed potato crops after which samples were incubated for 72 h in Pectate Enrichment Broth. During mechanical haulm destruction, samples were found positive with TaqMan assays specific for *Pectobacterium brasiliense* (%) and *P. parmentieri* (%). Air samples collected during plant growth before haulm destruction were all negative in the TaqMan assays, also samples collected during selection and after spraying chemicals. The application of metagenomics was subsequently explored on 12 of these air samples. In metagenomics, samples are analysed in a unbiased way and allow to discriminate pathogens at the strain level. Total DNA was extracted from the enriched air samples and sequenced (HiSeq). Sequences were mapped against reference genomes of soft rot *Pectobacteriaceae*. The presence of *P. brasiliense* and *P. parmentieri* was confirmed, but also *P. polaris*, *P. carotovorum* and likely *P. odoriferum* were detected for which no species specific TaqMan assays are developed yet. We further found strong indications that in samples different variants of *P. brasiliense* can be present. The use of metagenomics in diagnostics, in particular in forensic studies will be discussed.

This project was financed by the Dutch Ministry of Economic affairs via the TopSector Programme and the Dutch seed potato industry via the NAO

Prospects on host specificity and differential pathogenicity of *Pectobacterium* spp.

Nirmal Khadka[1,2], and Iris Yedidia[2]*

[1]Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel;

*[2] Institute of Plant Sciences, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel; *email: irisy@volcani.agri.gov.il*

With the "reputation" of a wide-host-range pathogen, specificity or "affinity" to a certain host is relatively a less studies aspect of *Pectobacterium* infection. Most microbial ecology studies of the genus were based on crop plants (vegetables or ornamentals) with potato being the most funded and studied system. In the last decades, with an ever improving genetic tools for phylogenetic classification, an increasing number of new species and subsp. in the genus were discovered. Despite all these tools, little attention has been devoted to the performance of different species on their respective hosts, their interaction with the host environment on a biochemical level, and the differential host responses towards different strains of *Pectobacterium*. Here, we characterized a differential response of isolates from potato (*P. carotovorum*, and *P. carotovorum* subsp. *brasiliense*) or calla lily (*P. aroidearum*) on two hosts: *Brassica oleracea* (cabbage) and *Zantedeschia aethiopica* (calla lily). The results revealed clear differential expression of virulence determinants within the bacterial strains in response to host's extracts; these were related to each strain's competence on its respective host. A similar trend of differential gene expression was demonstrated in the induced- resistance response of the plant hosts to the different bacterial strains.

Compatible mixtures of antagonistic bacteria developed specifically for biological control of Soft Rot *Pectobacteriaceae* (*Pectobacterium* spp. and *Dickeya* spp.) in potato (project PATBIOCON)

Dorota M. Krzyzanowska [1]§, Tomasz Maciag[1] §, Joanna Siwinska [2], Marta Krychowiak [3], Sylwia Jafra [1] and **Robert Czajkowski [3] ***

[1]*Laboratory of Biological Plant Protection,*

[2] *Laboratory of Plant Protection and Biotechnology,*

[3] *Laboratory of Biologically Active Compounds,*

Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Gdansk, Poland

§ - *these authors contributed equally to this work*

**Email: robert.czajkowski@biotech.ug.edu.pl*

Potato (*Solanum tuberosum* L.) is the fourth main food crop in the world after rice, maize and wheat. The very intensive global potato cultivation together with the international potato tuber market results in the risk of transmission and spread of various diseases. Diseases may influence potato production in any stage of crop growth and storage. Likewise, the diseases may affect tubers, above-ground plant parts (foliage) or both. From all diseases affecting potato, the ones caused by pectinolytic Soft Rot *Pectobacteriaceae* (SRP: *Pectobacterium* spp. and *Dickeya* spp.): blackleg of stems during plant cultivation and soft rot of tubers in storage are among the most important bacterial potato diseases recognized in potato production.
These diseases led to estimated losses of ca. 10 to 40% crop (even up to ca. 250 million Euro) annually worldwide. At the moment there are no commercial control products to be used against pectinolytic *Pectobacterium* and *Dickeya* in agriculture. This is due to the fact that during infection the bacteria are readily present in protective niches (inside plant vascular tissues)
and the majority of chemicals and physical control measures work only superficially and cannot penetrate to the tissues located deeper inside plants. The PATBIOCON project aims to use biocontrol measures to protect agricultural and ornamental plants from infections with pectinolytic bacteria. For this, we have selected beneficial bacteria occupying the same niche and antagonistic to *Pectobacterium* and *Dickeya* and evaluated them individually and
in compatible mixtures against the pectinolytic bacteria under disease-provoking conditions (high temp., high humidity, high pathogen load, hypoxia). Application of a combination
of (compatible) beneficial bacteria with different modes of antagonistic action provided significant protection of plant tissues against *Pectobacterium* and *Dickeya* (reduction of tuber soft rot by 46% ($p=0.0016$)). The selected antagonists were additionally characterized

for features important for their viable commercial applications including growth at different temperatures, resistance to antibiotics and potential toxicity towards *Caenorhabditis elegans*. The implications for control of soft rot caused by SRP with the use of the composition (mixture) of antagonists will be discussed.

The mixture of bacterial isolates described herein, for protection of potato tubers and ornamental plants against soft rot caused by pectinolytic Pectobacterium spp. and Dickeya spp., is the object of the patent application P.423806, which has been filed with the Polish Patent Office by University of Gdansk, Poland with inventors Robert Czajkowski, Dorota M. Krzyzanowska, Tomasz Maciag, Joanna Siwinska and Sylwia Jafra

The work was financially supported by the National Centre for Research and Development, Poland (Narodowe Centrum Badań i Rozwoju, Polska) via a research grant LIDER VI (LIDER/450/L-6/14/NCBR/2015) to Robert Czajkowski.

Direct current atmospheric pressure glow discharge in possible agricultural applications

Motyka-Pomagruk A[1*] Dzimitrowicz A[2] Jamroz P[2] Babinska W[1] Pohl P[2] Lojkowska E[1] and Sledz W[1]

[1]*Department of Biotechnology, University of Gdansk and Medical University of Gdańsk, Gdansk, Poland*

[2] *Department of Analytical Chemistry and Chemical Metallurgy, Wroclaw University of Science and Technology, Wroclaw, Poland*

**Email agata.motyka@biotech.ug.edu.pl*

Diseases caused by phytopathogenic bacteria are responsible for significant economic losses. Therefore high interest is attributed to development of novel eradication methods, not only to be applied in the fields but also in greenhouses, storage areas or having in mind decontamination of waterways or post-industrial wastes. For the latter purpose we herein propose a direct current atmospheric pressure glow discharge (dc-APGD)-based liquid sterilisation system (Polish patent application no. P.419246), utilization of which by generation of UVA, UVB and UVC in addition to the production of ROS and RNS (NOx, NH, H_2O_2, O_2, O and OH) led to complete eradication of *Clavibacter sepedonicus* IFB9034, *Dickeya solani* IFB0099, and *Xanthomonas campestris* pv. *campestris* IFB 9022 from suspensions of $OD_{600} \approx 0.1$ and at least 3.43 logarithmic reduction in the population densities of *Pectobacterium atrosepticum* IFB5103 and *Pectobacterium carotovorum* subsp. *carotovorum* IFB5118. Besides, dc-APGD was also applied for the synthesis of silver nanostructures (AgNPs), stabilized either by pectins (PEC), fructose (FRU) or sodium dodecyl sulphate (SDS). The obtained PEC-AgNPs and SDS-AgNPs exhibited antibacterial properties against *Dickeya* and *Pectobacterium* spp strains with minimal inhibitory concentrations (MIC) of 5.5 $mg \cdot L^{-1}$ and 0.75–3 $mg \cdot L^{-1}$, respectively. On the other hand, FRU-AgNPs were used against *Erwinia amylovora* IFB9037, *Clavibacter michiganensis* IFB9038, *Ralstonia solanacearum* IFB8019, *X. c.* pv. *campestris* IFB9022 and *D. solani* IFB0099 strains, showing MICs of 1.64-13.1 mg L^{-1} depending on the species tested. We postulate that dc-APGD might find numerous applications in the agricultural sector contributing to improvement of plant disease control.

Plant pathogen removal from tile drainage water by managed aquifer recharge for safe reuse as irrigation water in salinized agricultural areas

Eisfeldt, Carina[a], Van der Wolf, Jan M.[b], van Breukelen, Boris. M.[a], Schijven, Jack F.[c], Medema, Gertjan[a]

[a] *TU Delft, Faculty of Civil Engineering and Geosciences, Building 23, Stevinweg 1, 2628 CN Delft, The Netherlands*

[b] *Wageningen Plant Research, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands*

[c] *Utrecht University, Faculty of Geosciences, Heidelberglaan 2, 3584 CS Utrecht, The Netherlands*

The presence of bacterial plant pathogens in waterways and their dissemination through irrigation water pose a threat to crop production. A natural solution to provide safe irrigation water is managed aquifer recharge (MAR) for agriculture. Here, tile drainage water (TDW) is collected and infiltrated in brackish aquifers, resulting in a fresh water 'bubble' in the subsurface which gives farmers access to sufficient fresh water to irrigate their crops even in times of drought. The TDW may contain plant pathogens which could be present in the recycled water. To prevent the occurrence of crop diseases, the removal of selected plant pathogens in TDW and during aquifer soil passage will be analysed. We focused on three plant pathogenic bacteria, namely *Ralstonia solanacearum*, *Dickeya solani* and *Pectobacterium carotovorum sp. carotovorum* that are all present in the Netherlands but also depict a worldwide problem as they cause high crop losses associated with immense economical costs. Their die-off in natural TDW was studied under representative aquifer conditions in microcosm experiments and at a starting concentration of 10^4 CFU/mL. First results under aerobic conditions show that all bacteria were not detected anymore in 0.1-ml samples within 14 days at 10 ^{0}C using viable cell counting, corresponding to 3 $\log_{10}$ reduction by die-off. *D. solani* and *P. carotovorum* were no longer detected within 6 days at 25 ^{0}C, but *R. solanacearum* was more persistent at 25 ^{0}C and was detectable in 0.1 ml up to 25 days. Together with results from soil column experiments and field tests, these data will be used to predict the fate of pathogens during MAR using quantitative microbial risk assessment. Guidelines will be developed to demonstrate the feasibility of MAR in agricultural settings, its potential to deliver safe irrigation water and thereby fighting water scarcity and preventing food losses.

Since July 2017 Carina Eisfeld is pursuing her PhD research within the project of AGRIMAR which stands for agricultural managed aquifer recharge. Her part in the project is to analyse the water quality regarding plant pathogens to prevent the spread of plant diseases and consequently the loss of food crops. The goal is to proof that MAR technology is a safe and sustainable process that fights water scarcity in saline delta regions and provides safe water for irrigation.

Evaluation of the resistance to soft rot of commercial potato varieties in Chile

Acuña I., Sandoval C., Mancilla S. and A. Bermúdez.

Institute of Agricultural Research INIA Chile

E-mail: iacuna@inia.cl

Blackleg and soft rot, caused by *Pectobacterium atrosepticum* and *P. carotovorum carotovorum*, is a very important disease in potato production in Chile. This disease is responsible of 5 to 20% of yield losses, according to seed quality, environmental conditions and variety susceptibility. The objective of this study was to determine the variety susceptibility to soft rot on potato tubers. Seventeen potato varieties were evaluated using half tuber inoculation method with 1X 10^8 cfu of *P. atrosepticum* or *P. c. carotovorum*. Inoculated tubers were incubated at 27°C for 96 hrs. Disease severity was estimated measuring the rotten tissue volume per tuber. Atlantic, Rayun, Cornado and Patagonia INIA were the most susceptible, while Infinity, Romano and Red Sonia were the most resistant.

Soft rot in potato crops. Can complex systems approaches help to reduce disease incidence?

Johannes Ransijn

Dutch General Inspection Service for Agricultural Seed and Seed Potatoes (NAK)

Email: j.ransijn@nak.nl

Infection of potato plants with soft rot causing pathogens (SRP's, *Pectobacterium* and *Dickeya* species), is generally assumed to come from infected seed tubers (vertical transmission). There are several hints that horizontal transmission between potato lots or between potato and non-potato hosts plays a bigger role in the spread of SRP's than previously thought. The presence of multiple SRP species and species complexes and the recent spread of a previously absent species are indicative for the complexity and dynamism of the disease system.

The potential power of epidemiological and complex systems approaches has hardly been used for investigation of the potato soft-rot disease system. We are only beginning to apply methodologies developed for plant, human and veterinary epidemiology on the extensive data from routine phytosanitary surveys from the Dutch seed potato certification programme. An outline for future research and preliminary results will be presented and discussed.

The main objectives of the epidemiological research on SRP's are:

1) To assess the relative contribution of different infection pathways and the survival of SRP's on infected potato lots

2) To infer the importance of risk factors contributing to the spread, survival and virulence of different SRP's

3) To guide field and laboratory experiments and generate data-based hypotheses on the epidemiology and ecology of SRP's.

4) To guide policies to reduce the incidence of softrot and blackleg in potato crops by, for example, agronomic measures, field selection or risk-dependent intensity of inspection

Computational design of *Pectobacterium* diagnostics

Pritchard L*, Humphris S and Toth I

James Hutton Institute, Invergowrie, Scotland, DD2 5DA

**Email Leighton.Pritchard@hutton.ac.uk*

We report on progress in 2018 in diagnostic qPCR amplicon and metabarcoding marker design for *Pectobacterium*, using the PDP software package. We have designed over 40 novel diagnostic primer sets, intended to be specific to species: *P. atrosepticum*, *P. brasiliense*, *P. wasabiae*, *P. parmentieri*, *P. oderiferum*, *P. polaris*, *P. betavasculorum*, and universal to the genus Pectobacteriaceae. We have determined new candidate primers that were absolutely specific to *P. atrosepticum* (2 primer sets), *P. brasiliense* (1 set), *P. betavasculorum* (4 sets), and *P. oderiferum* (2 sets) by validation against a panel of 171 bacterial isolates. We will describe new features and capabilities of the PDP design software, including the ability to design candidate metabarcoding markers for within-species diversity estimation.

Additional information

Transport

There is a direct train connection from Schiphol to the railway station in Lelystad. The travel takes about 50 minutes. The Apollo hotel in Lelystad (Agoraweg 11) is located at a walking distance from the railway station (6 minutes).

The train tickets can be bought at the ticket office at Schiphol airport or the ticket machine. Information is provided on the following website: https://www.rijdendetreinen.nl/en/information/buying-train-tickets-netherlands, In case you want to buy a ticket in advance, use the following link: https://www.ns.nl/en

On Thursday and Friday, the transport by bus from the hotel in Lelystad to the NAK and back is arranged. In the morning the bus will pick you up at the hotel at 8.30 h. The costs of the bus transport is generously paid by HZPC.

In case you miss the bus or come late. NAK is located at Randweg 14, 8304 AS Emmeloord, Nederland, T T +31 527 635 400. The mobile telephone number of Miriam Kooman is +31.651191133 and of Jan van der Wolf +31.6.10028194.

Lunch and workshop dinner

The lunch on Thursday and Friday and the workshop dinner at Thursday night are kindly offered by the NAK.

Saturday, sight-seeing Amsterdam.

a bike tour: https://a-bike.eu/product/amsterdam-sightseeing-bike-tour/?gclid=EAIaIQobChMImK_2r_-U3QIVCed3Ch1ASQLaEAAYAyAAEgKRpvD_BwE

b. a boat tour: http://classiccanalcruises.com/ (for those that are hesitating to bike in November in the Netherlands)

The costs will be between 20 and 30 euro and are at your own expense.

Both tours will end approximately 13.00 h. If people like to stay for the lunch, I'm sure that they find their way in the many interesting cafés and restaurants.

List of Participants

	First name	Surname	Affiliation	E-mail
1	Ivette	Acuna	INIA	iacuna@inia.cl
2	Marie-Anne	Barny	Sorbonne Université	marie_anne.barny@agroparistech.fr
3	Doretta	Boomsma	HZPC	Doretta.Boomsma@hzpc.nl
4	Greig	Cahill	SASA	Greig.Cahill@sasa.gsi.gov.uk
5	Jérémy	Cigna	FN3PT/RD3PT	jeremy.cigna@fnpppt.fr
6	Nicole	Cotte-Pattat	Insa Lyon	nicole.cotte-pattat@insa-lyon.fr
7	Robert	Czajkowsi	University of Gdańsk	robert.czajkowski@biotech.ug.edu.pl
8	Yeshitila (man)	Degefu	Natural Resources Inst. Finland	yeshitila.degefu@luke.fi
9	Carina	Eisfeld	TU Delft	Carina.Eisfeld@tudelft.nl
10	Joost	Gierkink	HZPC	joost.gierkink@hzpc.nl
11	Vladimir	Gorshkov	Kazan Scientific Center	gvy84@mail.ru
12	Jianjun	Hao	Maine	jianjun.hao1@maine.edu
13	Valérie	Hélias	FN3PT/RD3PT/INRA	Valerie.helias@fnpppt.fr
14	Sonia	Humphris	James Hutton Institute	Sonia.Humphris@hutton.ac.uk
15	Eef	Jonkheer	WUR	eef.jonkheer@wur.nl
16	Miriam	Kooman	NAK	m.kooman@nak.nl
17	Viola	Kurm	WUR	viola.kurm@wur.nl
18	Ewa	Lojkowska	University of Gdańsk	ewa.lojkowska@biotech.ug.edu.pl
19	Agata	Motyka-Pomagruk	University of Gdansk	agata.motyka@biotech.ug.edu.pl
20	Minna	Pirhonen	Uhelsinki	minna.pirhonen@helsinki.fi
21	Leighton	Pritchard	James Hutton Institute	Leighton.Pritchard@hutton.ac.uk
22	Johannes	Ransijn	NAJ	j.ransijn@nak.nl
23	Åsa	Rölin	Potatis Konsult	asa.rolin@potatiskonsult.se
24	Martzen	ten Klooster	HZPC	martzen.tenklooster@hzpc.nl
25	Wojciech	Śledź	University of Gdansk	wojciech.sledz@biotech.ug.edu.pl
26	Leah	Tsror	Volcani Agri	tsror@volcani.agri.gov.il
27	Frederique	van Gijsegem	Sorbonne Université	vangijse@agroparistech.fr
28	Theo	van der Lee	WUR	theo.vanderlee@wur.nl
29	Jan	van der Wolf	WUR	jan.vanderwolf@wur.nl
30	Robert	Vreeburg	NAK	r.vreeburg@nak.nl
31	Malgorzata	Waleron	University of Gdansk	malgorzata.waleron@gmail.com
32	Iris	Yedidia	Volcani Agri	irisy@volcani.agri.gov.il