Project Report

Monitoring *Dickeya* and *Pectobacterium* species in the High Grade seed potato production area of Finland

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	From MMM	9500€
	From Luke	4071€
Key words	*Dickeya solani*, *Pectobacterium* spp, blackleg, soft rot, seed potato, High Grade area, disease monitoring, disease survey, diagnostics	

1. Summary

Targets

The aim of this short term project was to ensure the continuity of the monitoring of *Dickeya* and *Pectobacterium* species on potato in the HG seed potato production region and support the active participation in the Euphresco Dickeya and *Pectobacterium* species ecological studies and diagnostic method development networking which has been going on since 2011 at Luke Oulu in close collaboration of European partners. It is particularly focused on the 2015 growing season monitoring of potato blackleg at the period of transition of the end of Euphresco II in 2014 and the beginning of the Euphresco III project (2016-2018) implementation plan..

Implementation

To achieve the goals depicted in this short project, frequent field visits were carried out in the municipalities of Tyrnävä and Liminka during the growing season to assess the blackleg situation in the season. During the survey potato plants with blackleg symptoms were collected and samples were either analyzed right away or stored frozen in -80 °C until analyzed. Determination of the species of the bacteria associated with the symptom was determined by diagnostic PCR method developed in house. Isolations of the bacteria were performed using the semi selective Crystal Violet Polypectate (CVP) Agar or the differential medium, NGM Agar (for *Dickeya* spp. only). Dissemination of the knowledge gathered during this and earlier studies were conducted through publication of an article in a scientific journal, professional newsletter, lectures at Euphresco meeting (abroad) and Perunatutkimuksen talvipäivät in Finland. The organizational work concerning the Test Performance Study (TPS) could not materialize .It was called off by the organizers. However, the TaqMan real time PCR method development work for selected species of *Dickeya* and *Pectobacterium* was independently performed at Luke Oulu laboratory. Results of the test study for three major blackleg bacterial species are presented. The development work and validation of the detection system are included in the 2016-2018 project plans and will continue in 2016. Weather data analysis of the 2015 growing seasons was conducted from the meteorological data obtained from nearby weather stations (Oulusalo and Muhos) in North Finland and rational conclusions on weather- species prevalence correlation has been drawn.

Evaluation of Results

The weather analyses of the 2015 summer in Finland revealed a glimpse of a unique phenomenon with respect to the nature and incidence of the potato blackleg and soft rot and the response of the *Dickeya* and *Pectobacterium* under changing weather conditions. It particularly demonstrated the aggressive and competitive nature of *D. solani* when conditions for growth and development are met. The 2015 survey result was educational in several ways and it particularly provided useful knowledge about the blackleg complex and risk level associated with the species *D. solani*. The swift response of *D. solani* to the warm August after record cold June and July signified the high destructive nature of the species even with short period of high temperature conditions and the

higher risk associated with planting contaminated seed potato. The information was significant input towards a consolidated effort of establishing risk evaluation and management and decision support for sustainable blackleg management. The survey results further confirmed previous assumptions and/or observations that *D. solani* is an aggressive species which develops and spread within fields in a very short period overtaking (dominating) the other species particularly under warm conditions. The observation is intriguing and opens a subject for further investigations on the mechanisms of competitiveness of the species in mixed infections or disease complex. A take home message to potato producers and researchers is that never plant seed potato contaminated with *D. solani*.

2. Methods and Implementation of plans

Sustainable potato production in Finland and the rest of the world, among other things, depends on the supply of healthy seed. The HG zones in Europe, if properly managed, will have much to offer. The implementation of the provisions and principles underlying the status which includes steps to maintain the high plant health level of the potato crop and prevention of the import of seed potatoes from areas where certain harmful organisms are present is very crucial. However, the enforcement of these rationales are often challenged by the free trade agreements and trade liberalization practices of the free market economy which made seeds and planting materials to transit international borders relatively faster and easier.

It is, therefore, very important to consistently monitor all the potential diseases beginning at the port of entry and in the production fields. In order to reinforce the effort of protecting the HG area and preventing the outbreak and spread of blackleg and soft rot in the important seed potato growing area of Finland, the MTT Oulu (now Luke Oulu) established the infrastructure and began the research & development and seed certification service program for seed potato companies and producers in the HG zone. Validated, rapid and sensitive and specific molecular detection methods for the currently known blackleg and soft rot bacteria have been in place and used in both research and services since more than 10 years. Research focused on the incidence, ecology and diversity of *Dickeya* and *Pectobacterium* species have been going on for over a decade.

Monitoring is the backbone any pest or disease management system. Disease comprises interactions of host, parasite and environment. Therefore, for preventing disease outbreaks, it is possible to monitor the disease itself, and any one or more of its components. In the yearly monitoring and survey of outbreaks of blackleg in the HG area, analysis of weather parameters of the locality have been included as an aid to establish weather-disease and weather species relationships. Yearly surveys and collection of potato plants from fields and plots with typical blackleg symptoms from around the municipalities Liminka and Tyrnävä have been conducted. In addition, Seed lots submitted for voluntary seed certification represent a significant share of materials of investigation for assessing the incidence of the different species in seed potatoes produced in Finland or introduced from abroad. The knowledge gathered during the decade of monitoring, detection and characterization of *Dickeya* and *Pectobacterium* species on potato have been disseminated to international scientific community, seed potato companies and producers and Agricultural Advisory

services such as ProAgria through publication of scientific articles in international journals and professional newsletters; lectures in workshops and seminars and consultations.

This report includes the work accomplished in the 2015 cropping season with the financial support from MMM approved especially for the continuity of the monitoring work and other development research especially in upgrading the detection of *Dickeya* and *Pectobacterium* from potato and for the continuation of the networking and collaboration in the Euphresco joint initiative.

The main work activities accomplished in the project are summarized as disease surveys, determination of incidence of species in the disease complex, isolation and preservation of bacterial culture stocks, developments of Real Time qPCR detection method, analysis of weather data, networking and dissemination of knowledge. The results obtained are reported hereunder.

3. Results

3.1 *Disease survey, pathogen identification and stock preservation 2015*

The actual disease survey is often launched in the last weeks of July. However, inquiries were conducted through frequent phone calls to potato farmers and§ seed potato companies from around the municipalities of Tyrnävä and Liminka. Until the end of July, almost no blackleg was reported by farmers. However, the hope of a blackleg free season was short lived following the exceptionally warm August. On the first week of August 2015 symptoms of blackleg began to appear and an outbreak of high magnitude was reported especially on two fields planted with high class seed (SE23) of same variety and same seed source. The two fields were rejected on an inspection carried out on 14th August 2014. About 200 plants with blackleg symptoms were collected from the two fields and other fields where blackleg was observed at various degrees of incidence and severity. Repeated samplings were done in two weeks interval until harvest. *D. solani* was the only species detected from all blackleg symptomatic potato plants from collected on 14th August 2015 from the two heavy outbreak fields and other fields where mild attacks were observed. Later in September, as the weather was getting relatively cooler, P. atrosepticum few cases of few cases of P. wasabiae were detected. The *D. solani* strains were isolated using a differential medium Nutrient Agar Glycerol Manganese (NGM) (Lee & Yu, 2006) as illustrated in the Figure 1 below. The Blue colonies signifies Indogoidine production characteristic to *Dickeya* spp. The system works well mostly from symptomatic and heavily infected potato plants. It is less successful for the isolation of the bacteria latently present in the plant or tuber.

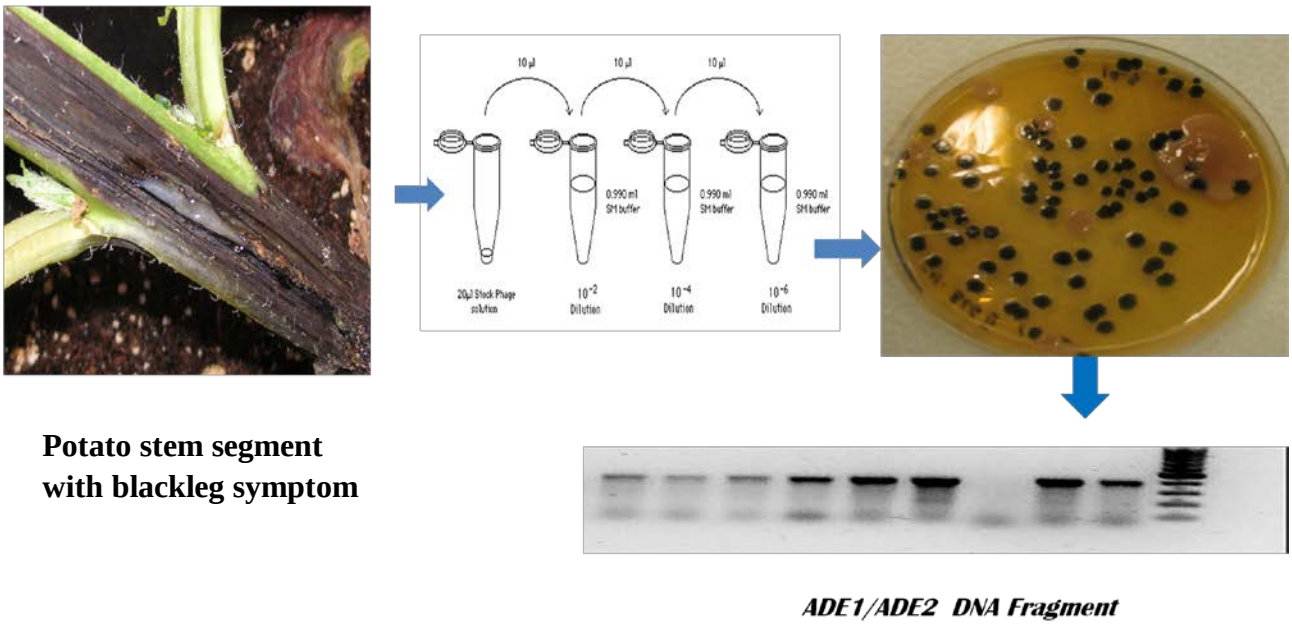

Figure 1. Detection and Isolation of *Dickeya* spp from infected and symptomatic potato plant on a differential medium (NGM Agar). Blue colonies signifies Indogoidine production characteristic to *Dickeya* spp. The system works mostly from symptomatic heavily infected potato plants. It is less successful for the isolation of the bacteria latently present in the plant. The isolates were first screened for Dickeya spp using the generic primers (ADE1 & ADE2) further identified as *D. solani* by Sol-C species specific primers.

Fifty *D.solani* isolates were maintained as glycerol stocks in -80 ºC and some isolates have been sent, upon request, to other Euphresco partners (for example to INRA, France) for characterization and sequencing. One other *D. solani* from our previous collections which displayed unique property of low proteases activity and lower virulence is under genome sequencing program at Ewa Ljokowska lab at the University of Gdansk in Poland through a collaboration created by the Euphresco joint initiatives.

3.2 Analysis of weather data and blackleg incidence in 2015 cropping season.

According to the 2015 weather statistics report by the 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. Furthermore 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), a condition exceptionally favorable for *D. Solani*. The last time that August was the hottest month of the summer was in 2006.

Table 1.Monthly temperature sum for the 2015 cropping season in the HG area

	MUHOS, Matokorpi	MUHOS, Matokorpi	MUHOS, Matokorpi	Oulunsalo
	2013	2014	2015	2001-2010
May	214	138	108	122
June	334	257	190	255
July	311	418	278	372
August	289	316	288	306
September	157	126	152	138

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 the two separate fields cropped with of the same variety and the same seed lot background. *Dickeya solani* was the gamer changer. This scenario unfolded because of the drastic weather changes in the month of August characterized by temperature conditions which especially favor *D. solani*. According to the general observation as many as five days of about 25 ºC or above 20 ºC are needed for *D.solani* to develop into an outbreak proportion and cause significant losses. This finding as it is evidenced in the Figure 2 confirms that general assumption.

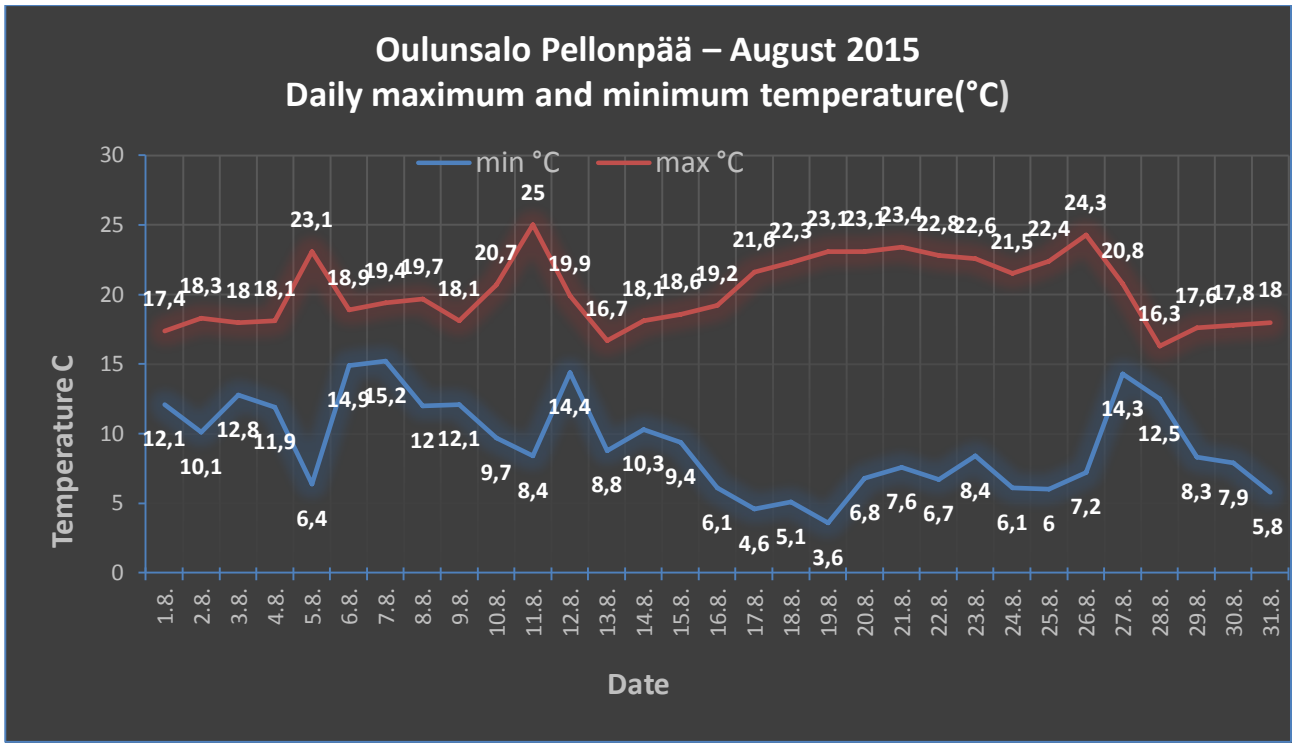

Figure 2. Daily maximum and minimum temperature for the month of August 2015 from Muhos (Oulu region) meteorological station in the HG seed potato growing area.

3.3. *Soft rot in storage, packing & processing and supermarkets*

According to our general observation and the information obtained from ProAgria (Rahko, personal communication) the overall incidence of blackleg and soft rot on potato fields in Finland in 2015 cropping season was characterized as mild, except the few cases of *D.solani* outbreaks described earlier in this report. However, an increased problem of soft rot has been observed in storage and supermarket shelves. Some examples of advanced rotting in storage prior to packing and tubers on supermarket shelf are shown (Fig 3). The late occurrence of favorable temperatures for infection somehow encouraged the continuing expression of the disease in storage. Development in store of soft rot often result in higher losses since the producer accrued the cost of production, harvesting, transportation and storage.

A B

Figure 3. Post harvest development of soft rot of potato. Supermarket shelf (A) and potato packing facility (B). Tubers in B were washed and stored at a temperature of 8 C prior the packing and forwarding them to market. Cross contamination during the washing may aggravate the higher incidence of tuber rot

3.4. Development of Real time qPCR detection system

The plan to conduct the TaqMan Real time QPCR detections of *Dickeya* and *Pectobacterium* species in 20 different labs in Europe and Israel under a coordinated program called the "Test performance Study (TPS)" could not materialize due to some technical difficulties encountered at the organizing laboratory. It was then left up to the different labs to decide what to do. The change of plan indeed require additional unexpected costs of primers and probes which otherwise would have come with the kit which was supposed to be dispatched from the coordinating laboratory. Considering costs and time issues, it was decided to conduct the test for selected species project at the Luke Oulu molecular biology laboratory. TaqMan Real Time qPCR protocols for *D.solani*, and *P. atrosepticum* and a Syber Green Real Time qPCR for *Dickeya* spp. and *P.* wasabiae have been conducted. The tests for the remaining species namely *P. carotovorum, P. brasiliensis*, and possibly

D. dianthicola including the validation of the whole detection system will continue during the first year (2016) of the new Euphresco (2016-2018) project financed by MMM. The target is to establish species specific TaqMan detection system for diagnostics purposes for all the *Dickeya* and *Pectobacterium* species present in Finland. The Syber Green system will also be used as an aid, for example, in screening of isolate collections since it is relatively cheaper. The primers used in this study in the Syber Green system were specific enough to detect the target bacteria mixed with a background bacterial species, as illustrated in the amplification curves and melting curve analysis (Fig. 4) although some inconsistencies have been indicated with primers DF and DR in test involving the direct detection of the bacteria possibly latently present in tubers (Pirhonen, personal communication), the primer pairs worked well to detect *Dickeya* in mixed bacterial population after enrichment. These issues will be verified from more enriched sample studies from latently infected tubers checked by conventional or endpoint PCR.

Table 2. Primers and probes used in qPCR test

Assay & Specificity	Primer / probe sequence (5'-3')	References
Dickeya solani	GCCTACACCATCAGGGCTAT ACACTACAGCGCGCATAAAC FAM-AGGCCGTGCTCGAAATCC-TAMRA	Pritchard et al 2013
Pectobacterium atrosepticum	CGGCATCATAAAAACACGCC CCTGTGTAATATCCGAAAGGTGG FAM-CATTCAGGCTGATATTCCCCCTGCC-TAMRA	DeBoer & Ward 1995 adapted by John Elphinstone for real-time PCR (Brierley et al. 2008)
Dickeya spp.	AGAGTCAAAAGCGTCTTG TTTCACCCACCGTCAGTC	Laurila et al 2010
Pectobacterium wasabiae	CTATGACGCTCGCGGGTTGCTGTT CGGCGGCGTCGTAGTGGAAAGTC	Kim et al. 2012

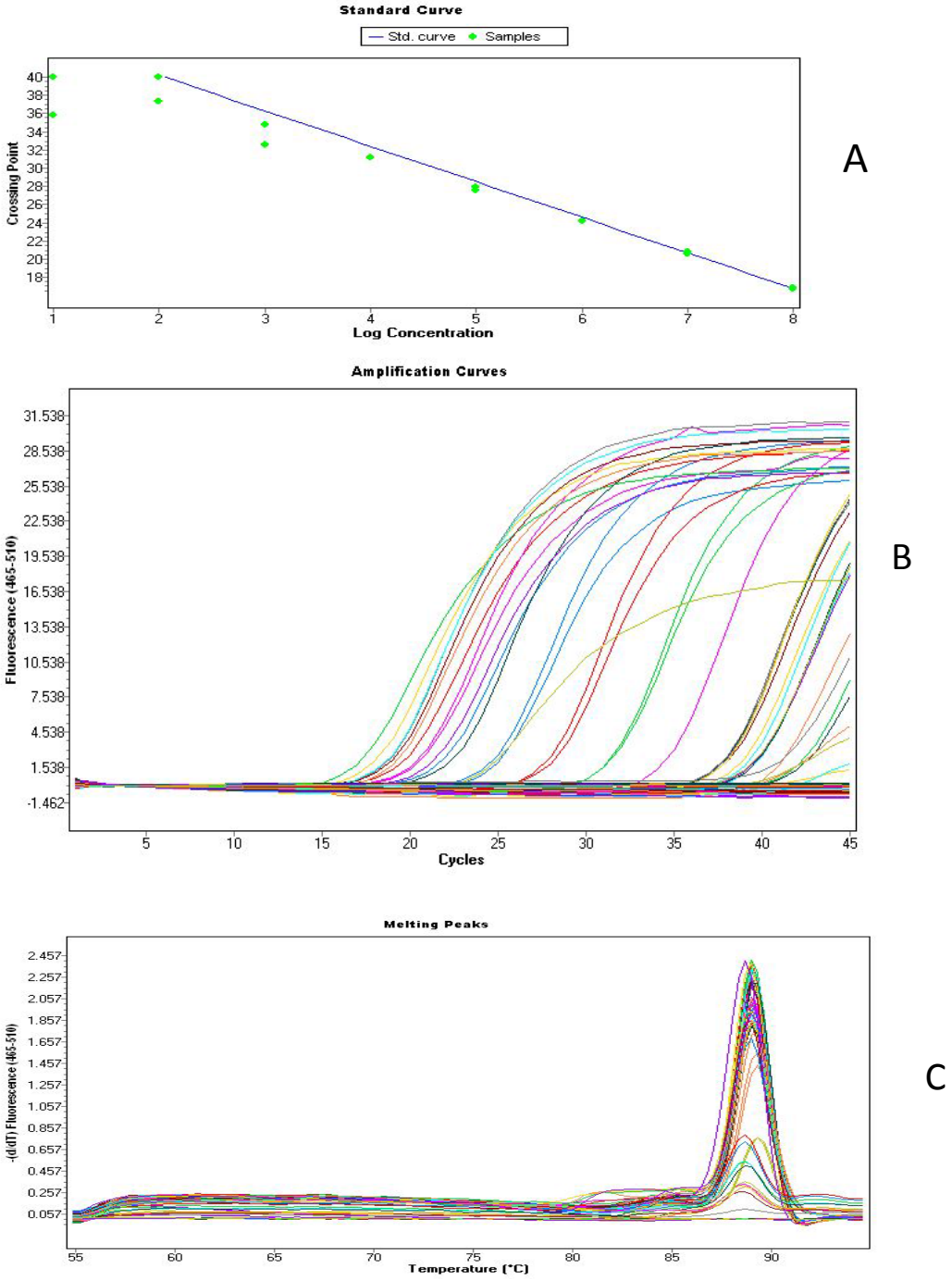

Figure 4. Real Time qPCR detection system of *Pectobacterium wasabiae* using the Syber Green chemistry. Figures represent values for the standards (A) amplification curves for all samples including controls, standards and unknowns (samples) (B) and melting curve analysis for all samples (C) showing a single peak demonstrating specific amplification of the target product.

The TaqMan Real time PCR assays for *D. solani* and *P. atrosepticum* also gave specific and reproducible results from repeated experiments involving a large number of target and non target bacteria (Fig.5).

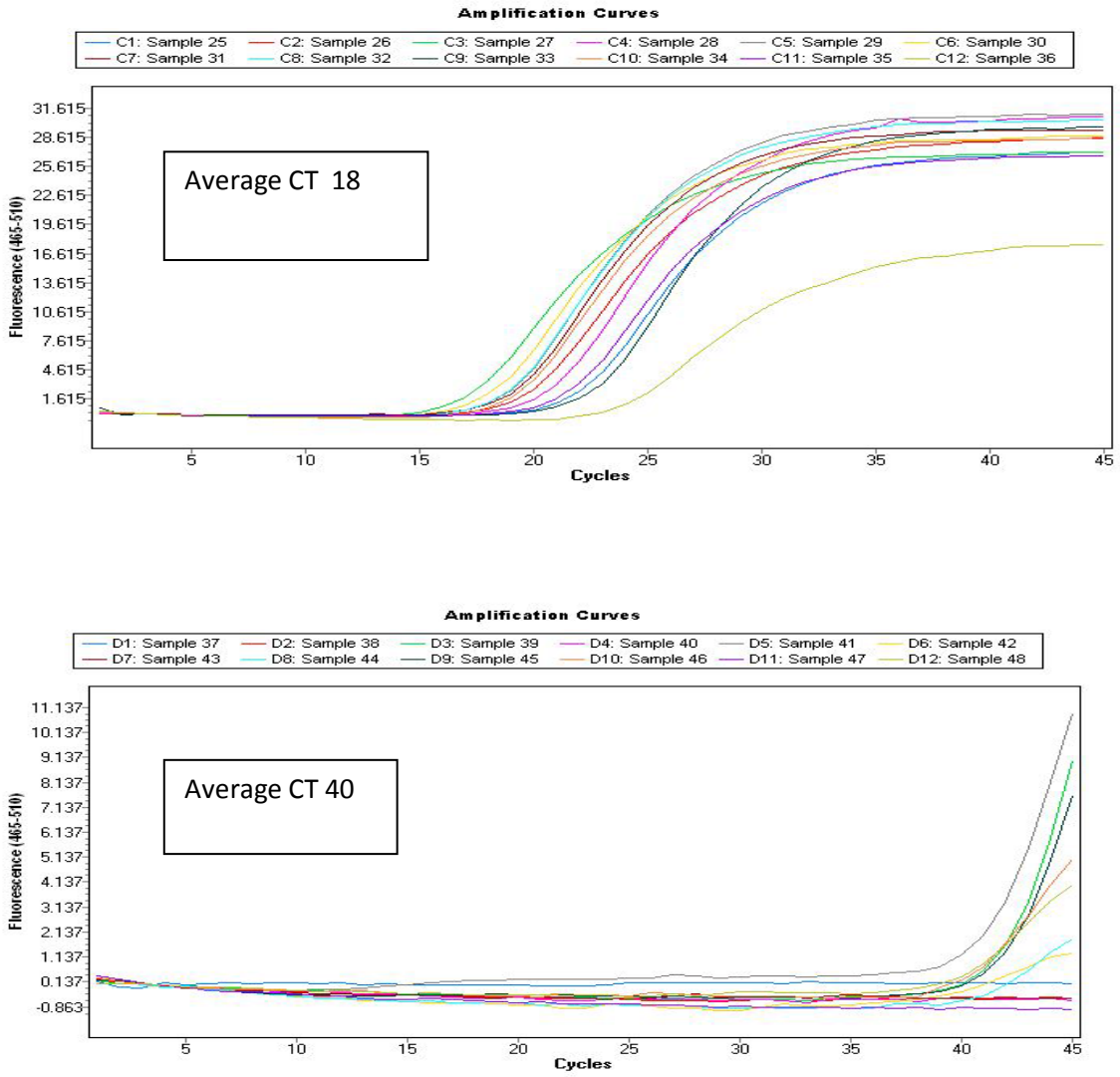

Figure 5. Detection of *Dickeya solani* using TaqMan Real Time qPCR assay (Pritchard et al. 2012) Amplification curves showing results (ct values) from positive sample (A) and negative controls (B). Negative controls include water controls and background non target bacteria.

4. Dissemination of knowledge

The knowledge gained in the previous and this study have been effectively communicated to domestic potato producers and researchers as well as European partners and to international scientific community at large through lectures and publications. The major undertakings are briefly highlighted below.

4.1 Publication;

Scientific article

Yeshitila Degefu**,** Panu Somervuo, Marja Aittamaa, Elina Virtanen, and Jari PT Valkonen. **Evaluation of diagnostic microarray in the detection of major bacterial pathogens of potato from tuber samples. EPPO Bulletin (in Press)**

Although the work on this article was conducted in previous years the final preparation and editorial processing work prior the acceptance of the manuscript were accomplished during this project time.

Presentations:

Presentation at the Euphresco III meeting Gdansk Poland (22.-24.11.2015)

***Dickeya solani,* the game changer in the 2015 outbreak of potato blackleg in Northern Finland.** For an audience of over 50 Euphresco *Dickeya a*nd *Pectobacterium* spp researchers from 17 member countries and participants from University of Gdansk, Poland.

Presentation at the Perunatutkimuksen talvipäivät. 20.-21.1.2016, Nokia, Finland

***Dickeya solani,* a game changer**. To audience of about 100 people (potato producers, researchers, potato processing companies, seed potato companies etc)

Presentation at Economic Commission for Europe Committee (UNECE) on Trade Working Party on Agricultural Quality Standards Specialized Section on Standardization of Seed Potatoes. Oulu, Finland, 8-11 September 2015

Blackleg/Soft Rot of Potato: A Disease Complex

(https://www.unece.org/fileadmin/DAM/trade/agr/meetings/ge.06/2015/ExtBureauMtg_Finland/GE_6_BUR_2015_z_Report_of_the_meeting.pdf)

Seminar to Plant and Animal Genetics Group (KMEG), Luke from Oulu to all members of KMEG in regional offices (toimipaikka) via Video *(October 27, 2016)*

Diagnostics of bacterial pathogens of potato in the genomic era: current status and future prospects.

5. Prospects

From historical records of Plant Pathology plant disease problems transcend geographical and boundaries and cause significant threats to humanity. Addressing such challenges in a sustainable manner requires strategic research, investments and international and regional collaboration, collective resource and knowledge exchange between diverse parties. The Euphresco project

initiative, in which this project form a part, is an example of such initiative towards achieving sustainable innovations for effective management of *Dickeya* and *Pectobacterium* spp on potato in Europe. The information gathered on the ecology and epidemiology, advances made on diagnostic tools and made available to research and services through more than a decade of monitoring, detection and characterization of *Dickeya* and *Pectobacterium* species in Finland (Degefu & Virtanen, 2015) has laid a good foundational basis for practical and sustainable solution to the consistent threat of *Dickeya* and *Pectobacterium* species on potato production (Degefu 2015)

6. References

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Lee YA, Yu CP (2006). A differential medium for the isolation and rapid identification of a plant soft rot pathogen, *Erwinia chrysanthemi*. *Journal of Microbiological Methods* 64:200-206.

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