

Fifteen years of monitoring, detection and characterization of soft rot and blackleg causing bacterial species of *Dickeya* and *Pectobacterium* in the High Grade seed potato growing area of Finland.

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Blackleg and Soft rot of Potato



The Research and development focus

- ❖ Finland as a country with High Grade (HG) status for seed potato production.
- ❖ Obligations and potentials of the HG
- ❖ Measures to minimize the introduction and establishment of new species of *Dickeya* and *Pectobacterium* in the country
- ❖ Detection and identification of *Dickeya* and *pectobacterium*
- ❖ Ecological studies (survival and overwintering)

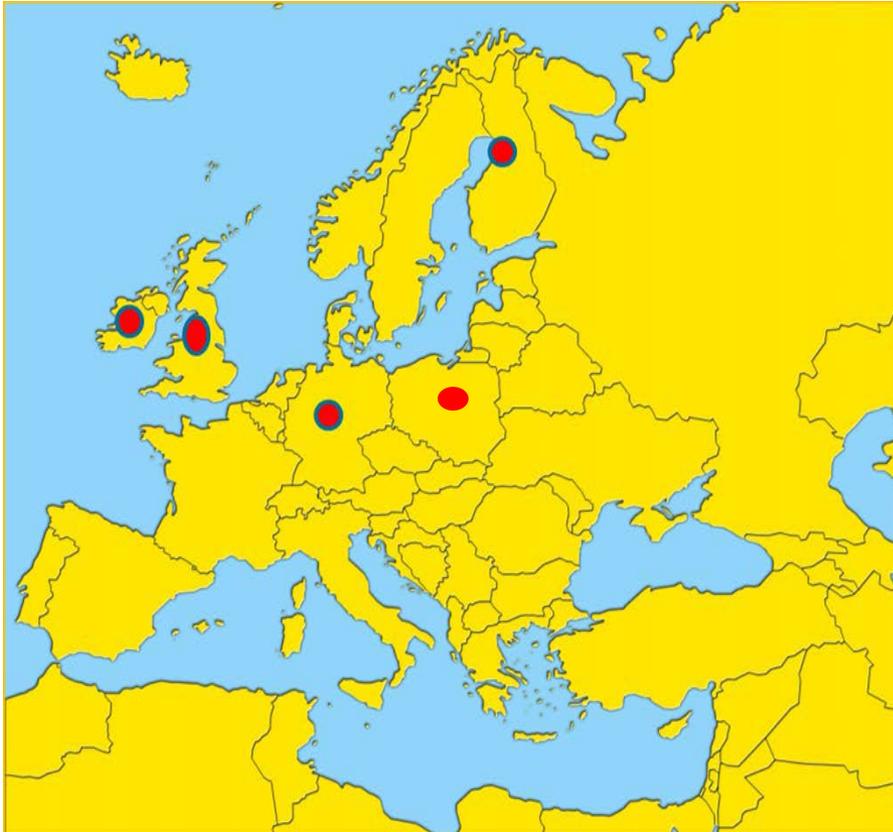
Major outcomes/Summary

- Provide a framework to study and understand the complexities of the disease and the causal agents
- Detection and identification
- Decision support system
- Measures to prevent establishment and spread of aggressive species especially in the High Grade (HG) area

Finland is the world's northernmost agricultural country. Finnish farmlands reach from the 60th latitude to north of the Arctic Circle (66° 33'47.8" north of the equator)



Finland is one of the **High Grade (HG)** seed potato production countries in Europe



Countries with HG status

- **North Finland**
- **Northern Ireland**
- **Scotland**
- **Cumberia and Northumberland in England**
- **Parts of Germany**
- **The Azores, Portugal**

Finland has been granted the HG status since 1995

EAPR Pathology and Pest Section meeting,
Neutachel, Switzerland

High Grade (HG) Finland: Oulu Region



**Municipalities of
Tyrnävä and Liminka**

**(65° 0' 45.414" N 25° 28'
17.231" E)**

High Grade(HG) status-The rationale

- **Free from harmful pests and diseases**
- **The right to establish stricter measures in the production and marketing of potatoes with a view to protect seed potato production in the area against organisms which may be particularly harmful to potato production**

What are the stringent measures in the HG area ?

- ✓ High class seeds E1, E2, E3
- ✓ Strong specialization in the production of seed potatoes
 - ✓ Seed potato : other potato production ratio
 - ✓ HG 1:1
 - ✓ Other parts of Finland 1:10

Cost

- **Maintaining the HG status costs 84,000€/ year to the state of Finland**

Benefits

- ✓ **Seed potatoes distributed to producers in Finland and marketed abroad are produced in the HG area.**
- ❖ **Northern Vigor™:** Northern Vigor™ in potato. Plants grown from seeds produced in northern hemisphere are more vigorous and are higher yielding than those grown using a southern seed source. This superiority was presumed to be due to reduced levels of seed borne disease and /or some inherent physiological characteristics of the seed tuber itself. **However, the exact reason(s) for this Northern Vigor™ is not known.**
- ✓ **Profitability of the Finnish seed potato industry.**

Challenges

The enforcement of these rationales are often challenged by

- **the existing free market economy and free trade agreements (trade liberalization policies)**
- **fast transportation system**
 - **allow planting materials to transit international borders relatively easier and faster**

There is a need for

**Research and disease
management tools in place**

Research and development objectives

Studies

- To ascertain **which bacteria are present in Finland**
- Access to an **accurate diagnostic tests** which include the **capability to distinguish important species** and subspecies of *Dickeya* and *Pectobacterium*.
- Sufficient **validation** of these tests to estimate their **reliability, sensitivity and specificity**
- Provide **Policy support systems** to review seed potato production guidelines and considerations for national and regional quarantine and biosecurity.

Research and Development

- **Building the infrastructure and expertise for research and services**
- **Constant monitoring of the introduction, establishment and prevalence of *Dickeya* and *Pectobacterium***
- **Detection and identification of the bacteria**
- **Seed testing and certification services**
- **Dissemination of knowledge and consultation**

DIAGNOSTICS

**RAPID, SENSITIVE AND
SPECIFIC Diagnostic
methods fundamental in
integrated Disease
management**

Important elements for predictive ability

- Sensitivity and specificity
- Discrimination between Viable & Dead pathogen
- Multiplexing (as many pathogens/reaction)
- Short diagnosis time (speed)
- High throughput
- Quantification
- Robustness
- Validation and standadization

Technology: Sensitivity and specificity

The advances in genomic sequencing created the possibility for designing a more versatile, robust as well as specific diagnosis of plant pathogens.

Technology: Detection of live bacteria-The dead has no meaning in diagnostics

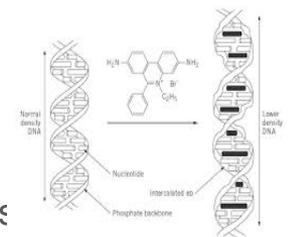
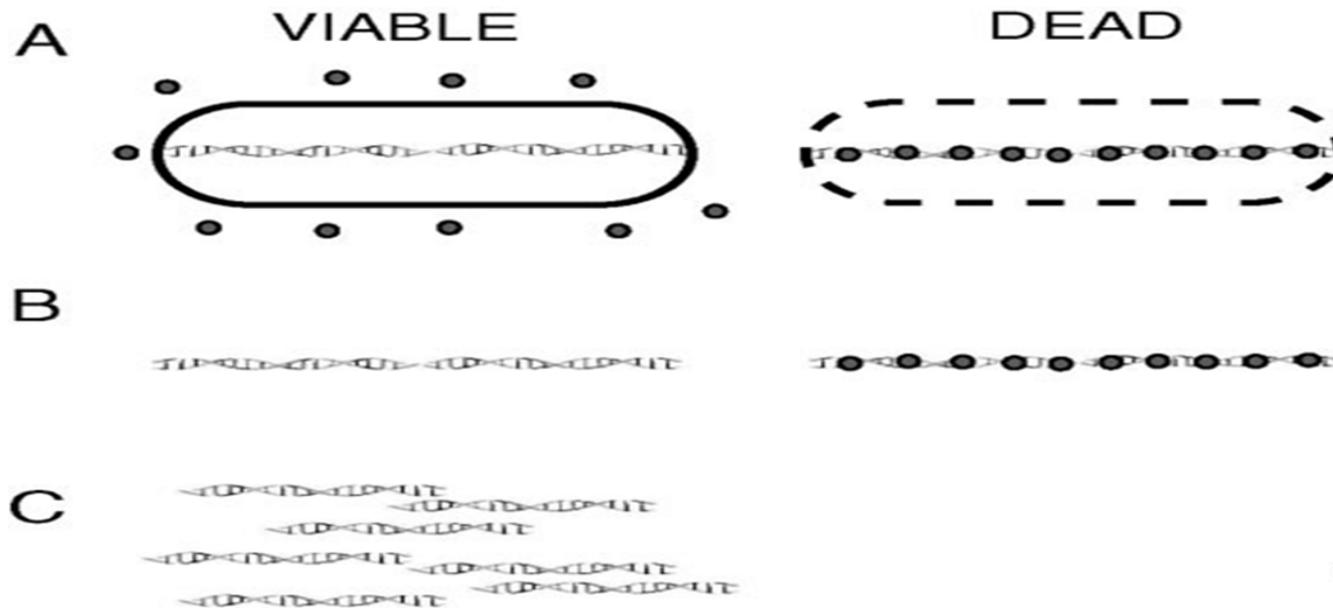
- ✓ **Enrichment culturing (BIO PCR)**
- ✓ **Use of RNA**
- ✓ **Ethidium Monoazide (EMA)**

Detection of live bacteria

Ethidium Monoazide (EMA) prevents PCR amplification from dead bacteria.

(Rudi et al. 2005, Appl Environ Microbiol. 71:1018-24)

Discrimination between live and dead cells in PCR by Ethidium Monoazide (EMA)



The DNA binding dye, Ethidium Monoazide (EMA) selectively penetrates dead cells (since dead cells have compromised plasma membrane) and intercalates into DNA upon photo-activation

(Rudi et al. 2005, *Appl Environ Microbiol* 71:1018-24)

Quantification: Better control of things we can quantify!

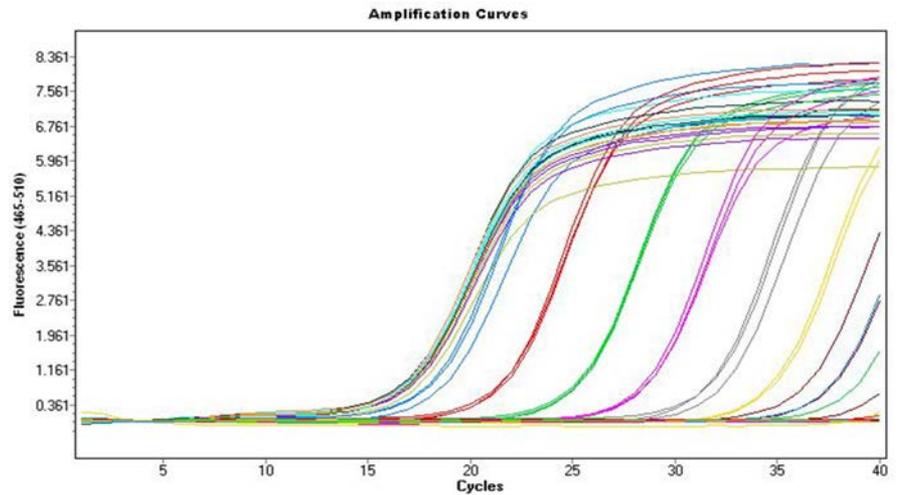
- Improved decisions support and risk management
- Threshold and tolerance limits.
- Disease management decisions and risk evaluation
 - **Bacterial number :disease correlation. According to one study HARD to establish.**

Detection & Quantification: QPCR



Light Cycler® 480 II

Degefu, Luke Oulu lab.



Microbial Detection Microarrays

Potential

- a platform of unlimited multiplexing capability
- opportunity for increased detection specificity through designing specific probes (complete genome sequence)

Drawback

- requires large amount of nucleic acid (problem in detecting latent *Dickeya* and *Pectobacterium* directly from tubers)
- need for preamplification of target (additional work flow)
- Cost

❖ **Hype than opportunity**

Ref: Degefu et al. 2016. EPP0 Bulletin

MONITORING: *Dickeya* and *Pectobacterium*

Transformation in the **aetiology of blackleg and soft rot in Finland**

- **From a serotype of one species to multiple taxa.**
 - **Development in diagnostic technology**
 - **Emmerging new species**

AETIOLOGY:

Species of *Dickeya* and *Pectobacterium* found on potato showing typical blackleg/soft rot symptoms in Finland

- *Pectobacterium atrosepticum*
- *Pectobacterium brasiliense*
- *Pectobacterium carotovorum*
- *Pectobacterium parmentieri*
- *Dickeya solani*
- *Pectobacterium polaris* (?)

▪ **Maybe more.....**

EAHP Pathology and Pest Section meeting,
Neutachel, Switzerland

Implications & observed consequences

- ❖ **Species are adapted to different temperature for growth and disease development**
 - **Likely occurrence of blackleg irrespective of the summer weather- cool or warm.**
- ❖ **Severe disease outbreaks especially when**
- ❖ ***D. solani* is involved (Degefu et al., 2013)**
- ❖ **Diagnostic complexity and high seed testing cost**

ECOLOGY

Survival:

Survival of *Dickeya* and *Pectobacterium* species in the environment outside the potato plant **is very poor or short** -

Pérombelon & Kelman, 1980, Degefu & Virtanen

(https://portal.mtt.fi/portal/page/portal/kasper/pelto/peruna/Potatonow/tutkimus/Yeshitila_PotatoNow_Article.pdf)

Soil (short survival)



Soil samples collected from heavily infected fields:

- From around infection foci while the potato is still in the field (**detected**)
- A week after harvest (**detected**)
- Spring soon after snow melted and before planting season started (not detected)

Weeds:

Survival in weeds uncertain



Bacteria not isolated from the roots of the suspected crucifer weed. No sign of infection.

Overwintering tubers: Bacteria do not survive on tubers exposed to cold Finnish winters



Tubers brought to the surface during land preparation and exposed to winter cold and snow cover were collected from several fields where heavy outbreaks of blackleg were recorded during the previous growing season.
NONE OF THE BLACKLEG AND SOFT ROT BACTERIA WERE DETECTED

Volunteer potato



Pathogen detected from daughter tubers resulting from mother tubers from previous season deep buried in soil.

Concluding Remarks- 1

Disease Diagnostics: dynamic and evolving.

”We need better methods for diagnosis: none of the methods given are to be considered as 'standardized' to think of them in such a way would put an end to efforts of improvement. They are useful only until better procedures can be developed” (Riker & Riker, 1936)

Introduction to research in plant diseases- a guide to the principles and practices for studying various plant disease problems)

Concluding Remark- 2

Blackleg/ soft rot is a disease complex (syndrome), its diagnosis and control seems rather complex

Concluding Remark 3: FIRST thing FIRST

Healthy and physiologically sound seed is fundamental for sustainable potato production. Therefore, start healthy (use certified seed) and remain healthy (consistent disease monitoring surveillance)

Concluding remark 4: Impact the seed potato center

- **Dissemination of knowledge at grass root level**
- **Consultancy services for the grass root**
- **Recognize the role of farmers in the diagnostic scheme (sampling) as an important element- **Representative sample****

Concluding Remark 5: Never be over confident

Worth remembering the saying by Ricker and Ricker back in 1936.

”We need better methods for diagnosis: none of the methods given are to be considered as ‘standardized’ to think of them in such a way would put an end to efforts of improvement. They are useful only until better procedures can be developed”

Thank you for your attention

