Diagnosis and Detection of *Dickeya* and *Pectobacterium*

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Wisconsin Seed Potato Certification Program
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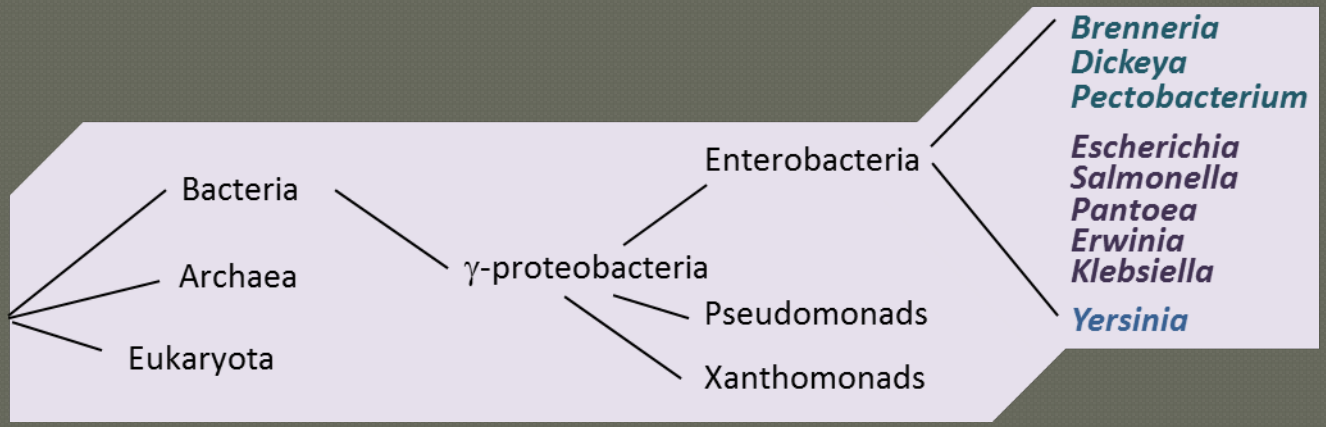
- Grouped as pectolytic *Enterobacteriaceae*



- Renamed:
  - *Erwinia carotovora = Pectobacterium*
  - *Erwinia chrysanthemi = Dickeya*

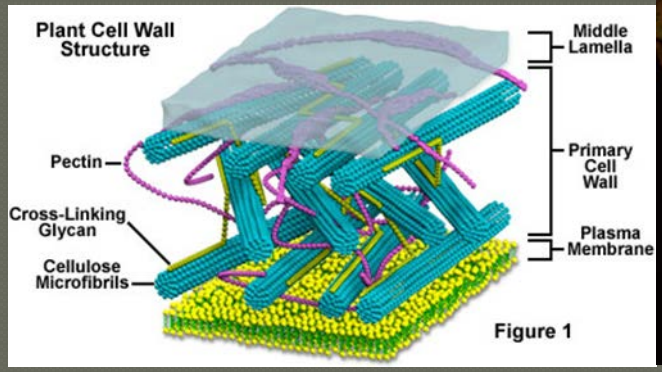
- Have a wide host range
  - Carrots, corn, broccoli, sunflowers
  - Does not thrive on legumes or small grains

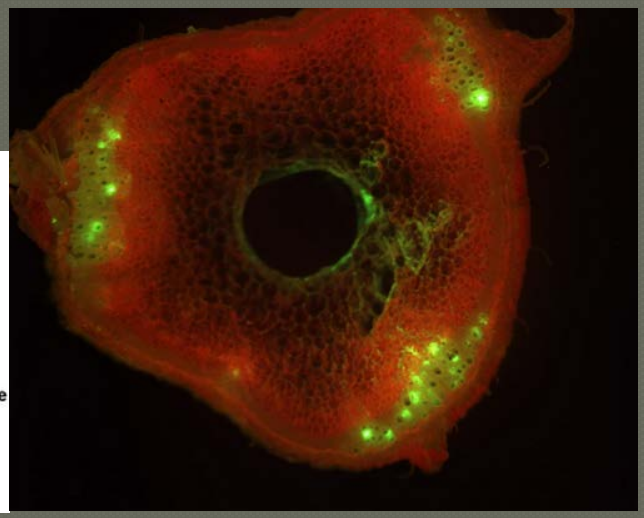
Dickeya and *Pectobacterium* Symptoms

- Cause symptoms by digesting plant cell walls
  - Seed piece decay
  - Blackleg
  - Stem rot
  - Tuber soft rot

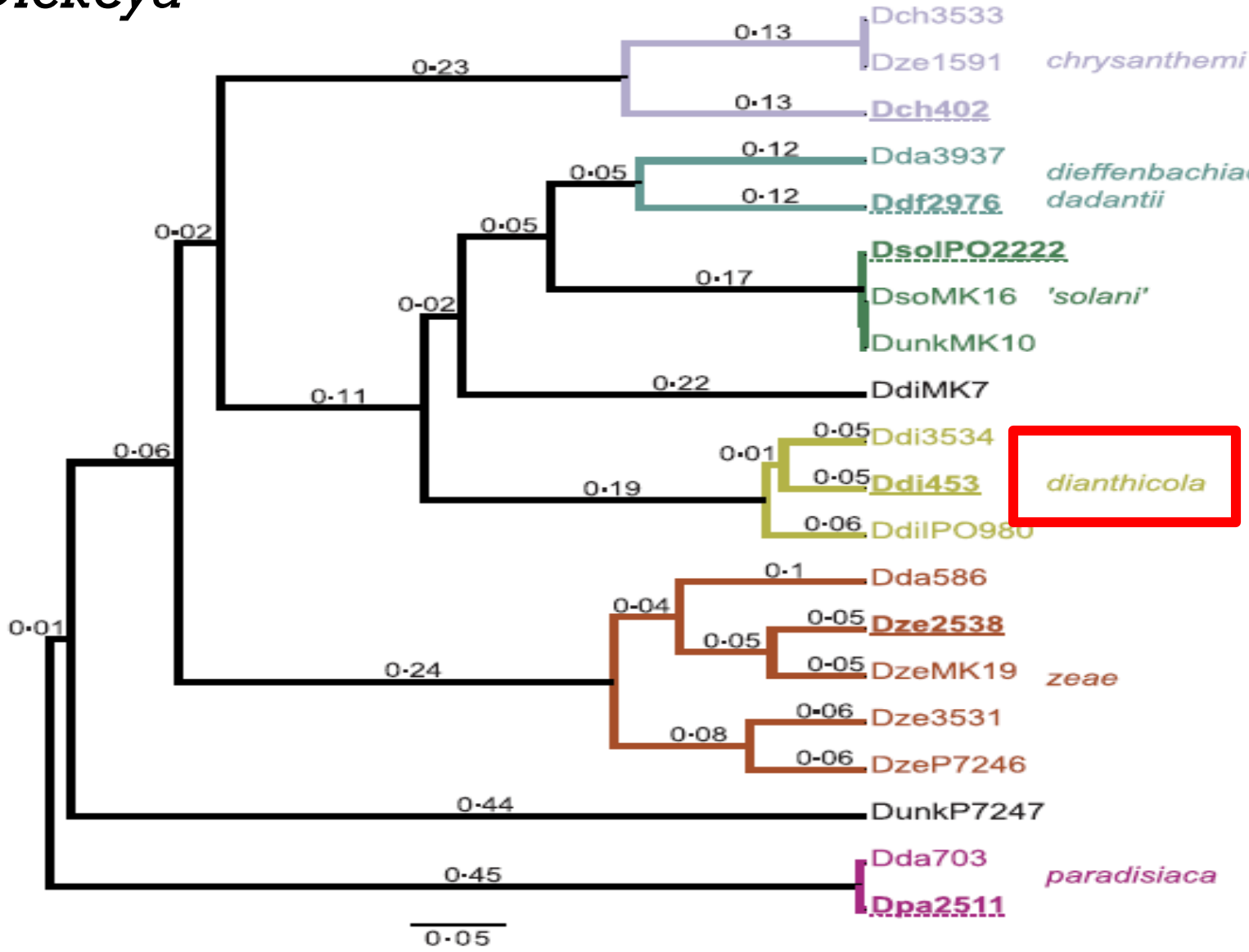
Symptoms are caused by enzymes that break down plant cell walls

- Bacterial cells observed in xylem
- Major virulence factors are cell wall degrading enzymes
  - Pectinases
  - Methyl-cellulases
  - Xylanases
  - Proteinases





Dickeya



L. Pritchard et al. 2013

D. Dianthicola Outbreak

- First reported in Europe in early 1970's
- In 2015, reported in several US states
- Origin of outbreak is unknown
  - Possibly present on other crops in US for years
- In 2016, reported in ME, MN, MI, ND, ID, FL, NJ, TX, PA and Ontario
  - The list keeps growing!

What caused the 2015 outbreak?

- Most likely been present in seed for a few years
  - Movement of latently infected potato seed
- Rainy conditions in 2013 and 2014 favored spread
  - Lower temps =Latent pathogen
- Saw significant losses in 2015 due to high temps

Symptoms

http://potatoes.ahdb.org.uk/media-gallery/detail/13214/2639

Dickeya vs *Pectobacterium*

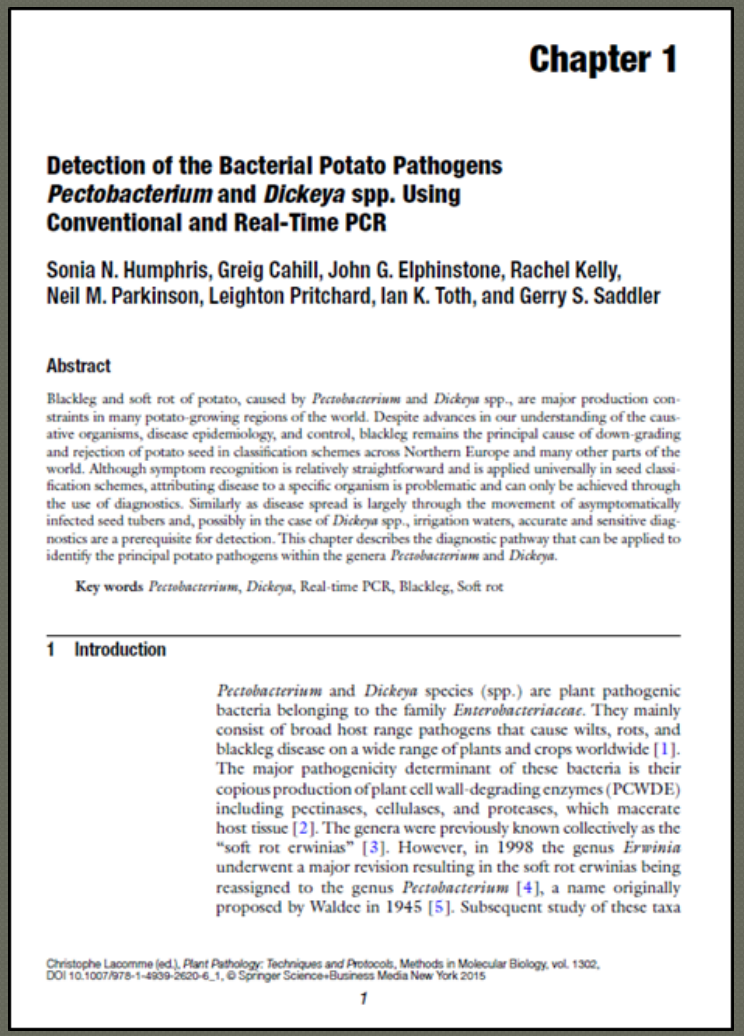
- Differ from *Pectobacterium*
  - More aggressive
  - Lower inoculum levels
  - Move easily through vascular tissue
  - Like warmer temps

  - Less likely to survive in soil

Detection of *Dickeya* and *Pectobacterium*

- PCR generally used for detection
- There are no PCR-based assays that can detect and differentiate all *Dickeya* and *Pectobacterium* species
- Genus-level detection is available
  - *Dickeya*-genus primers appear to work well
  - *Pectobacterium*-genus primers less reliable

Multiple options for PCR primers



Chapter 1

Detection of the Bacterial Potato Pathogens *Pectobacterium* and *Dickeya* spp. Using Conventional and Real-Time PCR

Sonia N. Humphris, Greig Cahill, John G. Elphinstone, Rachel Kelly, Neil M. Parkinson, Leighton Pritchard, Ian K. Toth, and Gerry S. Saddler

Abstract

Blackleg and soft rot of potato, caused by *Pectobacterium* and *Dickeya* spp., are major production constraints in many potato-growing regions of the world. Despite advances in our understanding of the causative organisms, disease epidemiology, and control, blackleg remains the principal cause of down-grading and rejection of potato seed in classification schemes across Northern Europe and many other parts of the world. Although symptom recognition is relatively straightforward and is applied universally in seed classification schemes, attributing disease to a specific organism is problematic and can only be achieved through the use of diagnostics. Similarly as disease spread is largely through the movement of asymptomatically infected seed tubers and, possibly in the case of *Dickeya* spp., irrigation waters, accurate and sensitive diagnostics are a prerequisite for detection. This chapter describes the diagnostic pathway that can be applied to identify the principal potato pathogens within the genera *Pectobacterium* and *Dickeya*.

Key words *Pectobacterium*, *Dickeya*, Real-time PCR, Blackleg, Soft rot

1 Introduction

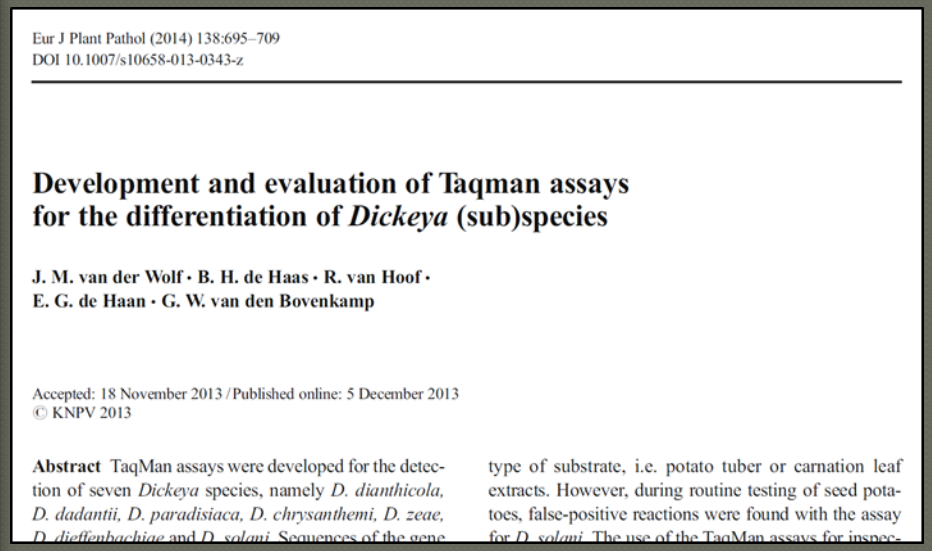
Pectobacterium and *Dickeya* species (spp.) are plant pathogenic bacteria belonging to the family *Enterobacteriaceae*. They mainly consist of broad host range pathogens that cause wilts, rots, and blackleg disease on a wide range of plants and crops worldwide [1]. The major pathogenicity determinant of these bacteria is their copious production of plant cell wall-degrading enzymes (PCWDE) including pectinases, cellulases, and proteases, which macerate host tissue [2]. The genera were previously known collectively as the "soft rot erwinias" [3]. However, in 1998 the genus *Erwinia* underwent a major revision resulting in the soft rot erwinias being reassigned to the genus *Pectobacterium* [4], a name originally proposed by Waldee in 1945 [5]. Subsequent study of these taxa

Christophe Lacomme (ed.), *Plant Pathology: Techniques and Protocols*, Methods in Molecular Biology, vol. 1302,
DOI 10.1007/978-1-4939-2620-6_1, © Springer Science+Business Media New York 2015

Humpris et al. provides the most comprehensive overview of *Dickeya* and *Pectobacterium* detection methods.

Christophe Lacomme (ed.), *Plant Pathology: Techniques and Protocols*, Methods in Molecular Biology, vol. 1302

Multiple options for PCR primers



Eur J Plant Pathol (2014) 138:695–709
DOI 10.1007/s10658-013-0343-z

Development and evaluation of Taqman assays for the differentiation of *Dickeya* (sub)species

J. M. van der Wolf · B. H. de Haas · R. van Hoof · E. G. de Haan · G. W. van den Bovenkamp

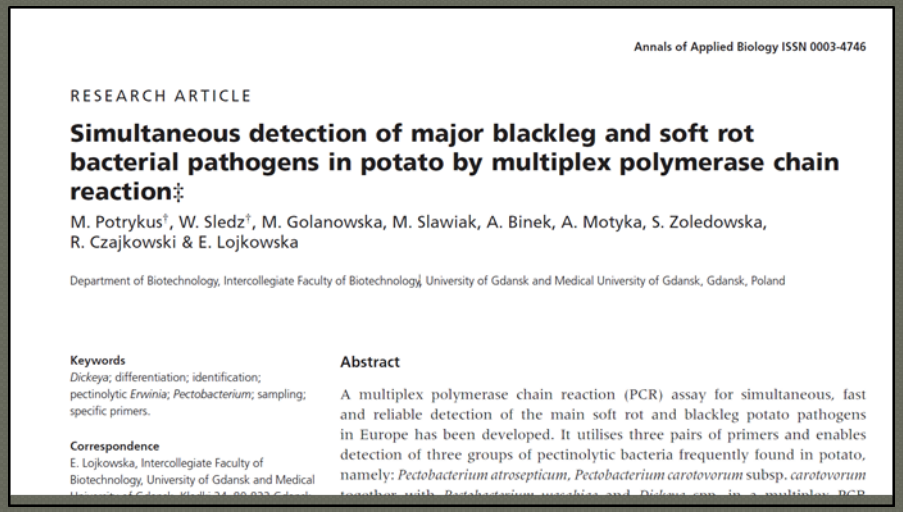
Accepted: 18 November 2013 / Published online: 5 December 2013

Abstract TaqMan assays were developed for the detection of seven *Dickeya* species, namely *D. dianthicola, D. dadantii, D. paradisiaca, D. chrysanthemi, D. zeae, D. dieffenbachiae* and *D. solani*. Sequences of the gene

type of substrate, i.e. potato tuber or carnation leaf extracts. However, during routine testing of seed potatoes, false-positive reactions were found with the assay for *D. solani*. The use of the TaqMan assays for inspec-

- Van der Wolf et al. (2014) designed primers to detect *Dickeya* species
- Most were not validated with field samples and those validated with field samples did not work well
- Ex: multiple false positives using a *D. solani* primer set

Conventional PCR-Multiplex Assay



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RESEARCH ARTICLE

Simultaneous detection of major blackleg and soft rot bacterial pathogens in potato by multiplex polymerase chain reaction‡

M. Potrykus†, W. Sledz†, M. Golanowska, M. Slawiak, A. Binek, A. Motyka, S. Zoledowska, R. Czajkowski & E. Lojkowska

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Keywords
Dickeya; differentiation; identification; pectinolytic *Erwinia*; *Pectobacterium*; sampling; specific primers.

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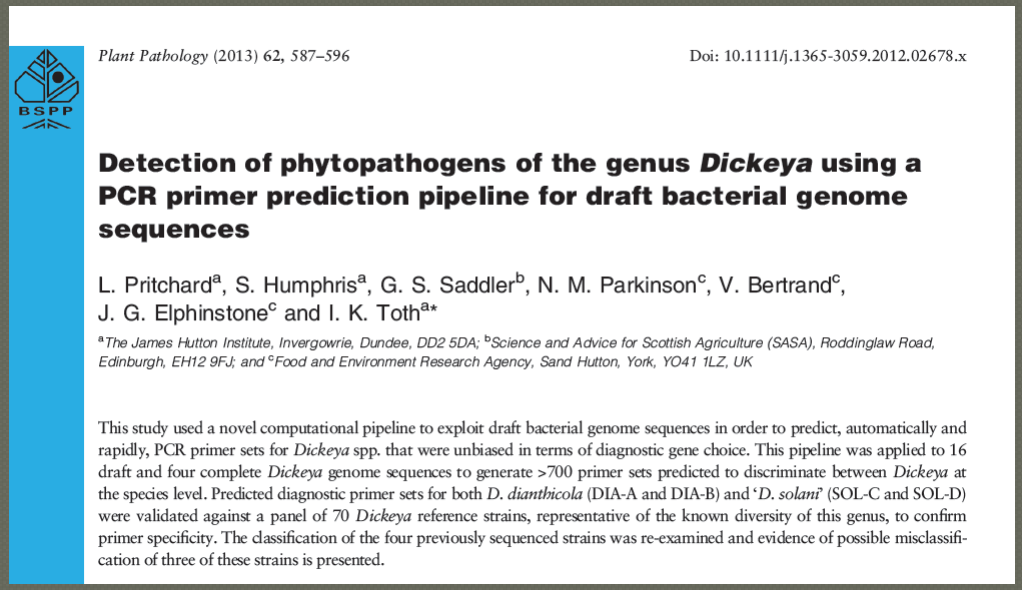
Abstract

A multiplex polymerase chain reaction (PCR) assay for simultaneous, fast and reliable detection of the main soft rot and blackleg potato pathogens in Europe has been developed. It utilises three pairs of primers and enables detection of three groups of pectinolytic bacteria frequently found in potato, namely: *Pectobacterium atrosepticum*, *Pectobacterium carotovorum* subsp. *carotovorum*

- Potrykus et al. (2014) combined three conventional PCR primers into a multiplex assay
- Genus *Dickeya* primer set (Dsp) appears to work well
- Two *Pectobacterium* species specific primers:
  - *P. atrosepticum* (Pba)
  - *P. carotovorum subsp. carotovorum* (Pcc)
- *Pectobacterium* primer sets do not work as well

Real-time PCR



Plant Pathology (2013) **62**, 587–596 Doi: 10.1111/j.1365-3059.2012.02678.x

Detection of phytopathogens of the genus *Dickeya* using a PCR primer prediction pipeline for draft bacterial genome sequences

L. Pritchard[a], S. Humphris[a], G. S. Saddler[b], N. M. Parkinson[c], V. Bertrand[c], J. G. Elphinstone[c] and I. K. Toth[a]*

[a]*The James Hutton Institute, Invergowrie, Dundee, DD2 5DA;* [b]*Science and Advice for Scottish Agriculture (SASA), Roddinglaw Road, Edinburgh, EH12 9FJ; and* [c]*Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, UK*

This study used a novel computational pipeline to exploit draft bacterial genome sequences in order to predict, automatically and rapidly, PCR primer sets for *Dickeya* spp. that were unbiased in terms of diagnostic gene choice. This pipeline was applied to 16 draft and four complete *Dickeya* genome sequences to generate >700 primer sets predicted to discriminate between *Dickeya* at the species level. Predicted diagnostic primer sets for both *D. dianthicola* (DIA-A and DIA-B) and '*D. solani*' (SOL-C and SOL-D) were validated against a panel of 70 *Dickeya* reference strains, representative of the known diversity of this genus, to confirm primer specificity. The classification of the four previously sequenced strains was re-examined and evidence of possible misclassification of three of these strains is presented.

- Pritchard et al. validated several primer and probes for *Dickeya*
- *Dickeya* primer set (ECH) appears to work well
- Probes available for:
  - *D. dianthicola* (DIA-A)
  - *D. solani* (SOL-C, D)

Testing

- Highest *Dickeya* concentration:
  - Stem end of tuber
  - Base of plant stems

- Soak tuber cores or stem tissue in water or ¼ strength Ringer's solution for 1 hr
  - Ringer's is an isotonic solution
- Observed bacteria levels are higher in stems

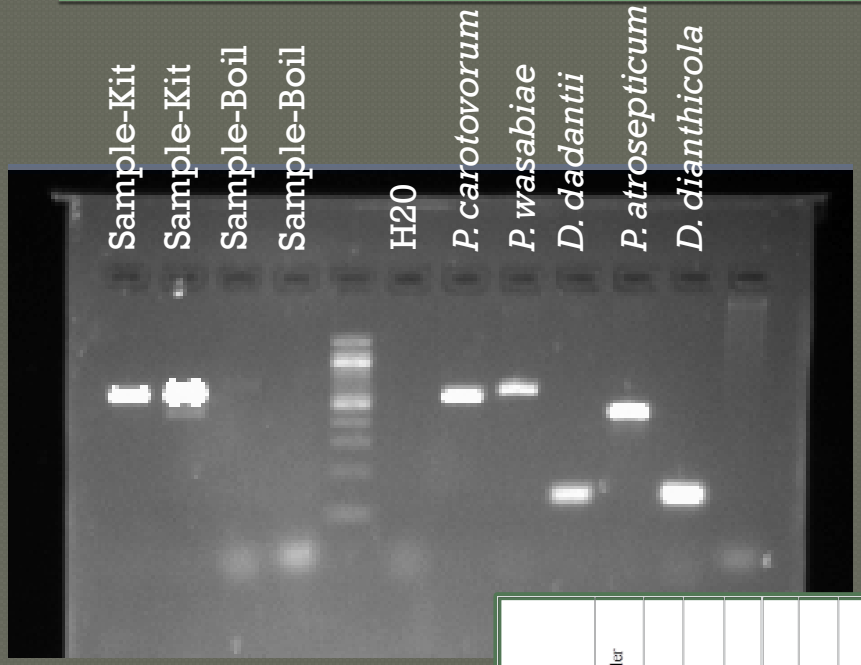
Testing

- DNA kits, such as the FastDNA SPIN kit for soil can be used to isolate DNA from stem or tuber core suspension

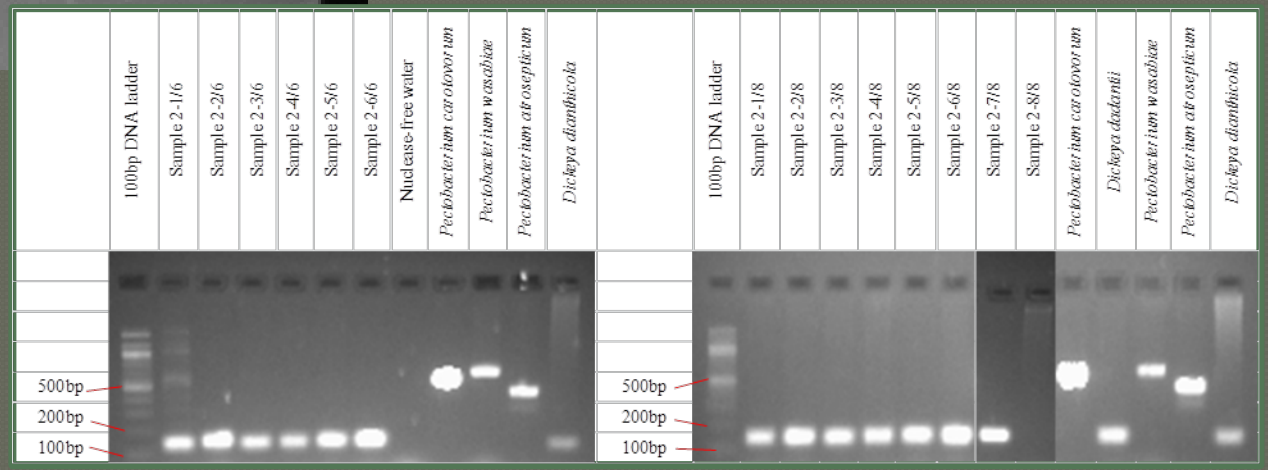
- Have had mixed results with boiling method

Example of Multiplex Assay

(Potrykus et al.)

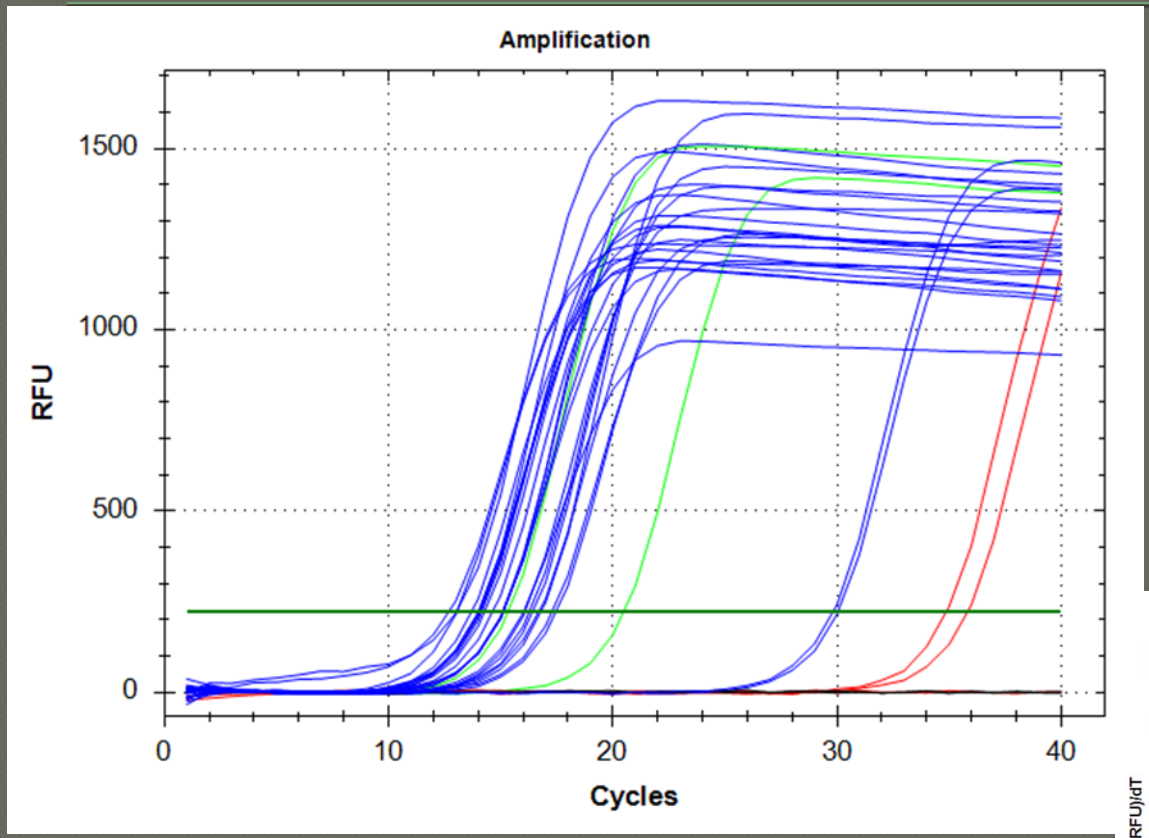


- Works well for *Dickeya* but challenging to determine *Pectobacterium* species

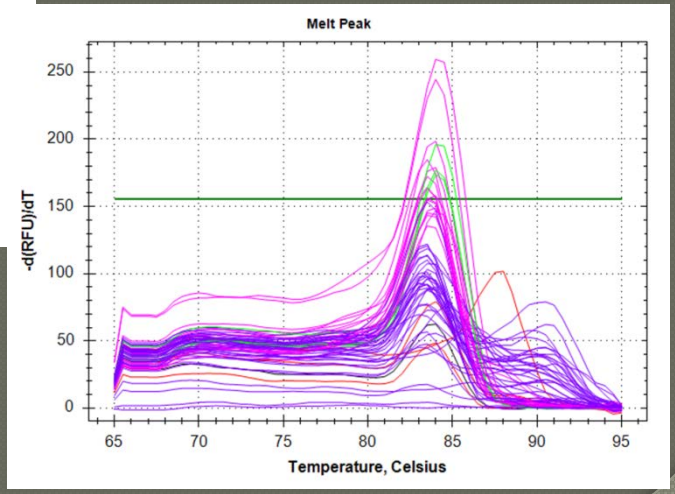


Example Real-time PCR

(Pritchard et al.)

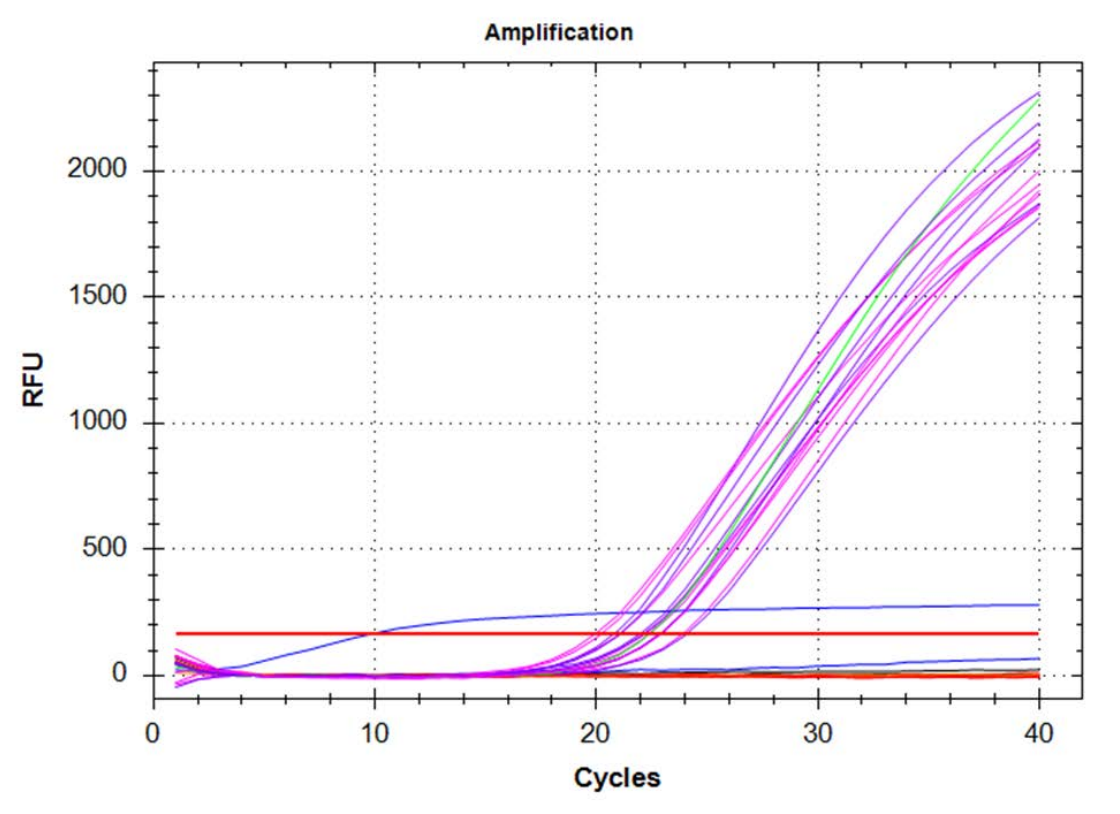


- Using ECH primer specific to *Dickeya*
  - SYBR Green
- See late amplification-analyzing melt curve data



Example of Real-time PCR

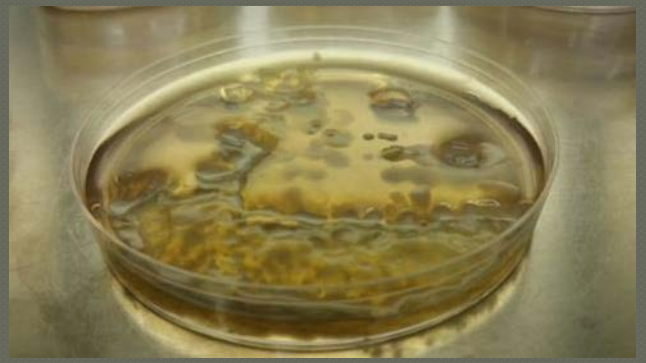
(Pritchard et al.)



- DIA-A probe with Texas Red
- Specific for *D. dianthicola*

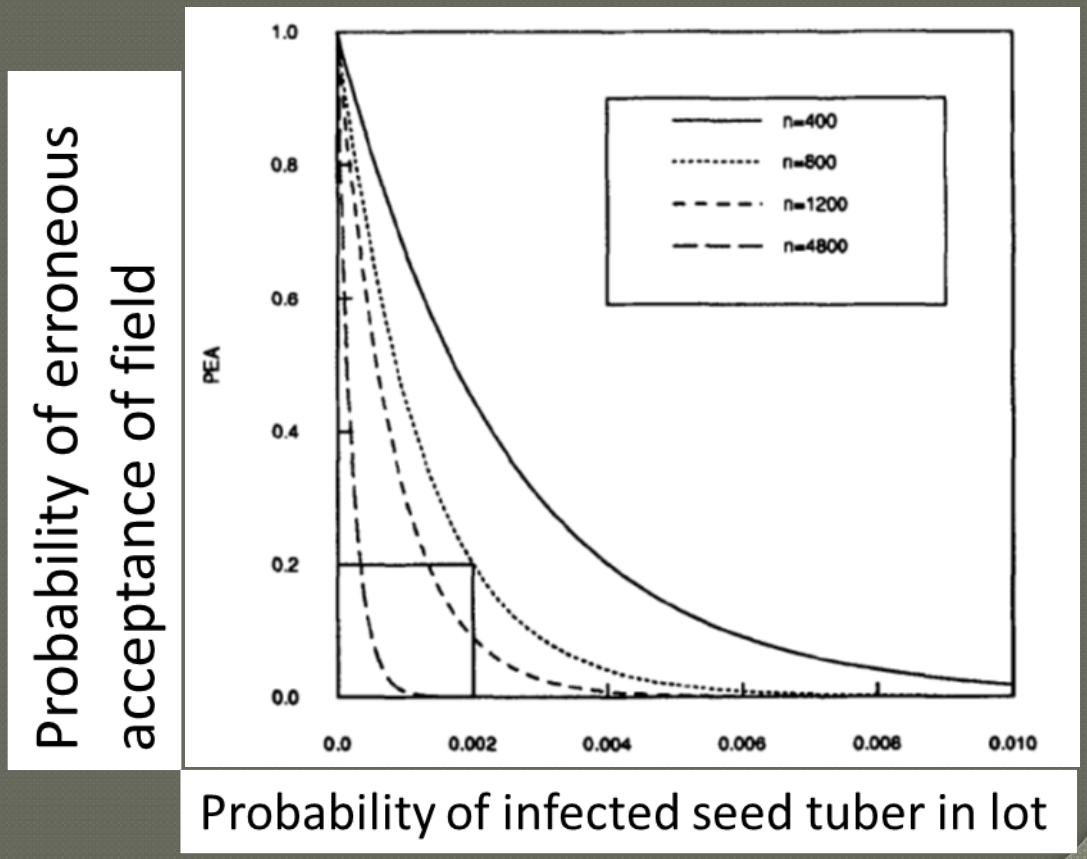
Plating

- Crystal violet pectate (CVP) works well for *Pectobacterium,* but seems to be less effective for *Dickeya*
- *Pectobacterium* grows well on LB and nutrient agar
- *Dickeya* does not survive very long on LB, but grows and survives on nutrient agar
- Hope to test additional types of media that are not pectate-based to improve *Dickeya* isolation.



Testing Seed lots for *Dickeya* and *Pectobacterium*

How many tubers should be tested?



Clayton and Slack. 1988. Amer. Potato J.

How many tubers should be tested?

- 400 tubers per lot = likely to identify seed lots with 1% or greater incidence
- 1200 tubers per lot = likely to identify seed lots with 0.3% or great incidence
- 4605 tuber = ~ 0% infection

http://labs.russell.wisc.edu/potato-blackleg/