Diagnosis and Detection of *Dickeya* and *Pectobacterium*

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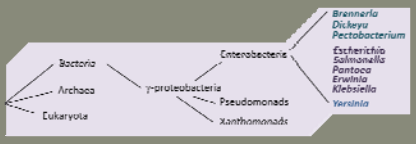
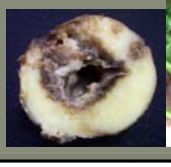
- Grouped as pectolytic *Enterobacteriaceae*

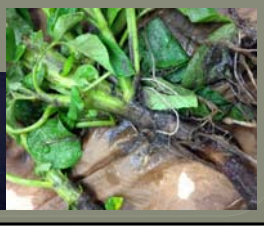


- Renamed:
  - *Erwinia carotovora* = *Pectobacterium*
  - *Erwinia chrysanthemi* = *Dickeya*
- Have a wide host range
  - Carrots, corn, broccoli, sunflowers
  - Does not thrive on legumes or small grains

Dickeya and *Pectobacterium* Symptoms

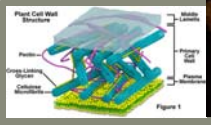
- Cause symptoms by digesting plant cell walls
  - Seed piece decay
  - Blackleg
  - Stem rot
  - Tuber soft rot

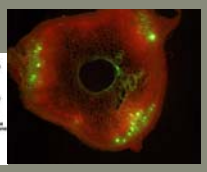




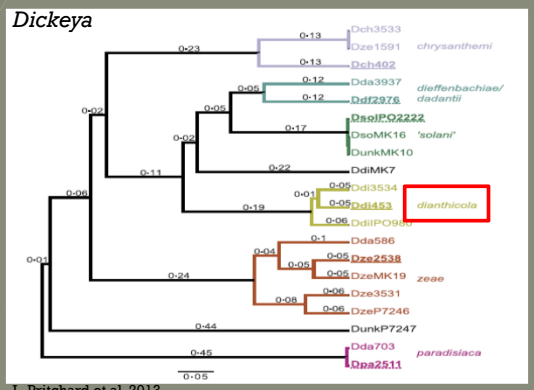
Symptoms are caused by enzymes that break down plant cell walls

- 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

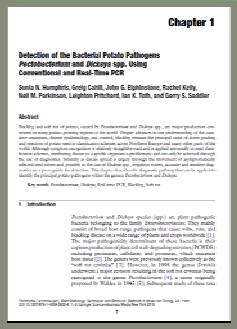
Dickeya vs *Pectobacterium*

- 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 Kirby, Neil M. Parkinson, Leighton Pritchard, Ian K. Toth, and Garry S. Saddler

Abstract

1 Introduction

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

Christopher Lamourex (ed.), Plant Pathology: Pathogenesis and Prophylaxis, Methods in Molecular Biology, vol. 1302,

Multiple options for PCR primers

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

- 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


RESEARCH ARTICLE

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

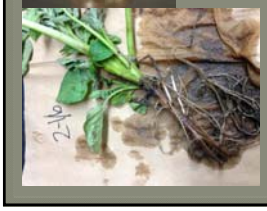
- Potrykus et al. (2014) combined three conventional PCR primers into a multiplex assay
- Genus *Dickeya* primer set (Dsp) appears to work well
- Two *Pectobacterium* species specific primers:
  - *P. atrosepticum* (Pba)
  - *P. carotovorum subsp. carotovorum* (Pcc)
- *Pectobacterium* primer sets do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

Real-time PCR


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

- 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



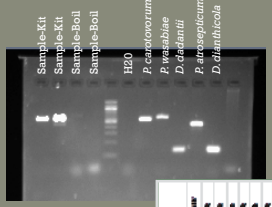
- Highest *Dickeya* concentration:
  - Stem end of tuber
  - Base of plant stems
- Soak tuber cores or stem tissue in water or ¼ strength Ringer's solution for 1 hr
  - Ringer's is an isotonic solution
- Observed bacteria levels are higher in stems

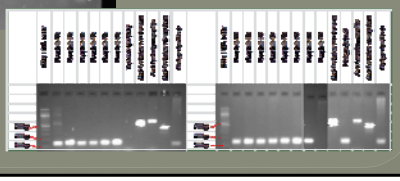
Testing

- 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.)

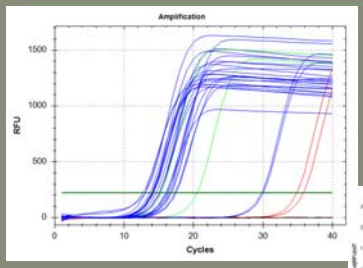


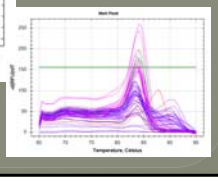


- Works well for *Dickeya* but challenging to determine *Pectobacterium* species

Example Real-time PCR

(Pritchard et al.)

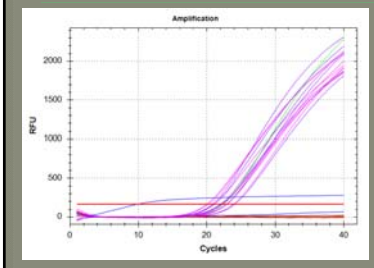




- Using ECH primer specific to *Dickeya*
  - SYBR Green
- See late amplification-analyzing melt curve data

Example of Real-time PCR

(Pritchard et al.)



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

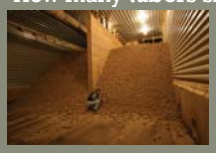
Plating

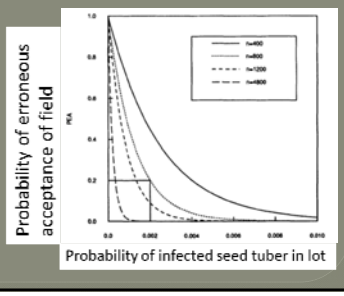
- Crystal violet pectate (CVP) works well for *Pectobacterium*, but seems to be less effective for *Dickeya*
- *Pectobacterium* grows well on LB and nutrient agar
- *Dickeya* does not survive very long on LB, but grows and survives on nutrient agar

- 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/