

## Diagnosis and Detection of *Dickeya* and *Pectobacterium*

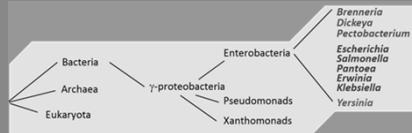


Brooke Babler

Wisconsin Seed Potato Certification Program  
UW-Madison



- 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

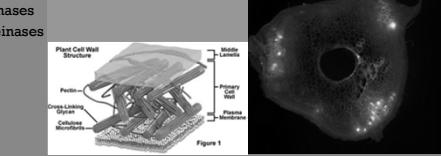
### *Dickeya* and *Pectobacterium* Symptoms

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

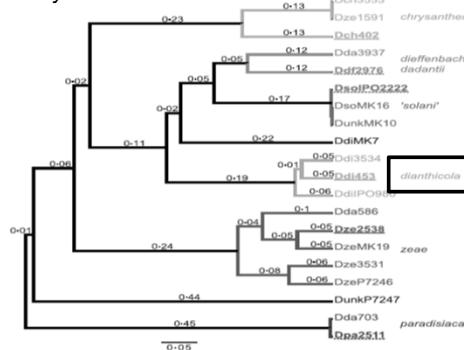


### Symptoms are caused by enzymes that break down plant cell walls

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



### *Dickeya*



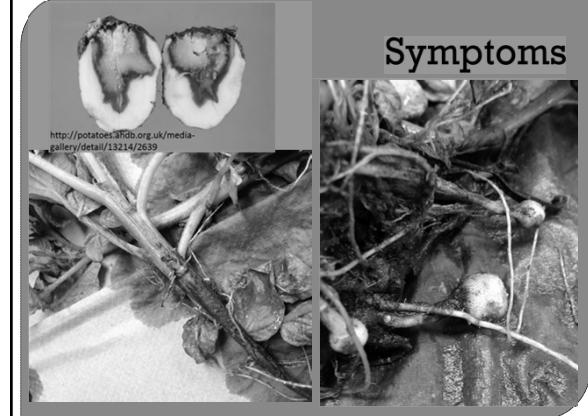
L. Pritchard et al. 2013

### *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



## *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., via more definitive conventional and Real-Time PCR**

Sonia N. Humphris, Gregor Caielli, John G. Eghball, Rachel Kelly, Neil M. Parkinson, Leonie Pritchard, Ian K. Tish, and Gerry S. Soderlind

**Abstract**

*Dickeya* and soft rot of potato, caused by *Pectobacterium* and *Dickeya* spp., are major pathogens causing significant economic losses to the potato industry worldwide. The ability to rapidly and accurately detect these pathogens is crucial for disease management and to prevent their spread. This chapter describes the molecular detection of these pathogens using conventional PCR and real-time PCR. The chapter also describes the development of genus-specific primers for *Dickeya* and *Pectobacterium*, including positive, negative, and process controls, which ensure the reliability of the assays. The chapter concludes with a section on the detection of *Dickeya* spp. in field samples, including a larger study involving the use of a cross-reacting primer set developed by Walker in 1981 [1]. Subsequent study of these samples revealed that the assay was able to detect *D. solani* and *D. chrysanthemi* but not *D. dipsaceum*. The chapter also describes the development of Taqman assays for the differentiation of *Dickeya* (sub)species.

**Keywords** Potato, *Pectobacterium*, *Dickeya*, Real-time PCR, Bacterial soft rot

**1 Introduction**

*Pectobacterium* and *Dickeya* spp. (spp.) are plant pathogenic bacteria that cause soft rot of potato and other solanaceous crops. These bacteria are among the most important causal agents of losses in potato production worldwide [1]. The major pathogenic determinants of these bacteria is their capacity to produce enzymes such as pectinolytic enzymes [2], including proteases, cellulases, and pectinases, which enable them to penetrate the plant cell wall and to move from one cell to another [3]. In addition, they are able to produce a range of toxins, including cytotoxins, that are involved in the pathogenesis of the disease [4].

**2 Materials and methods**

**2.1 Isolation of *Dickeya* spp. and *Pectobacterium* spp. from field samples**

**2.2 Preparation of DNA from field samples**

**2.3 Preparation of DNA from pure cultures**

**2.4 PCR amplification**

**2.5 Gel electrophoresis analysis**

**2.6 Real-time PCR**

**2.7 Taqman assays for the differentiation of *Dickeya* (sub)species**

**2.8 Statistical analysis**

**3 Discussion**

**4 Conclusions**

**References**

**Author information**

**Correspondence:** Sonia N. Humphris, School of Biological Sciences, University of Nottingham, Nottingham NG7 2RD, UK. (Email: s.humphris@nottingham.ac.uk)

Humphris 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

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

J. M. van der Wolf<sup>1</sup>, R. H. de Haas<sup>2</sup>, R. van Houdt<sup>1</sup>, E. G. de Haas<sup>1</sup> & G. W. van den Broekcamp<sup>1</sup>

Accepted: 18 November 2013; Published online: 1 December 2013

**Abstract** Taqman assays were developed for the detection of seven *Dickeya* species, namely *D. chrysanthemi*, *D. atroseptica*, *D. pectiniphila*, *D. dipsaceum*, *D. solani*, *D. corynorhini* and *D. carotovora*. The specificity of the assays was tested against a wide range of bacterial isolates, including *D. chrysanthemi*, *D. pectiniphila*, *D. dipsaceum*, *D. solani*, *D. corynorhini*, *D. carotovora*, *Pectobacterium* spp., *Erwinia* spp., *Agrobacterium* spp., *Phytophthora* spp., *Pythium* spp., *Sclerotinia* spp., *Botryotinia* spp., *Candidatus* *Mycobacterium* spp., *Yersinia* spp., *Escherichia* spp., *Shigella* spp., *Salmonella* spp., *Enteropathogenic Escherichia coli* and *Enterotoxigenic Escherichia coli*.

**Keywords** *Dickeya*, soft rot, potato, Taqman, PCR

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➤ 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

**Real-time PCR**

Plant Pathology (2015) 42, 187–196  
DOI: 10.1016/j.ypath.2015.02.004

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

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<sup>a</sup>The James Hutton Institute, Dundee, DD9 6HT, United Kingdom; <sup>b</sup>Ministry and Food Agriculture (M&F), Bern, Switzerland; <sup>c</sup>AgResearch, PO Box 31-0200, Private Bag 31-0200, Lower Hutt, New Zealand

The authors have declared that they have no conflict of interest.

This article is part of a Special Issue on *Dickeya* and *Erwinia* plant pathogen research. The review and discussion articles in this issue were submitted by invited authors. The editor would like to thank all the authors for their contributions to this special issue.

**> 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)**

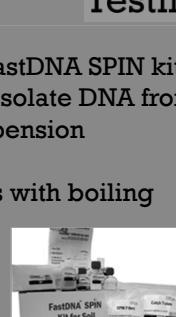


## Testing

- Highest *Dickeya* concentration:
  - Stem end of tuber
  - Base of plant stems
- Soak tuber cores or stem tissue in water or  $\frac{1}{4}$  strength Ringer's solution for 1 hr
  - Ringer's is an isotonic solution
- Observed bacteria levels are higher in stems



- 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., 2013)

## Example Real-time PCR

(Pritchard et al.)

The figure consists of two side-by-side line graphs. The left graph, titled 'Amplification', plots 'RFU' (Relative Fluorescence Units) on the y-axis (0 to 1500) against 'Cycles' on the x-axis (0 to 40). Multiple black lines show exponential growth in fluorescence, starting near zero and plateauing around 1500 RFU after approximately 25 cycles. A horizontal grey line is drawn at approximately 100 RFU. The right graph, titled 'Melt Peak', plots 'dF/F' (Fluorescence change) on the y-axis (0 to 200) against 'Temperature, Celsius' on the x-axis (65 to 95). Multiple black lines show a single peak that shifts to higher temperatures as the cycle threshold increases. A vertical grey line is drawn at approximately 100 dF/F.

## Example of Real-time PCR

(Pritchard et al.)

## 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/>

Thank you!