A TaqMan qPCR-based detection assay for *Pseudomonas syringae* pv. *aptata* causing bacterial leaf spot of Table beet and Swiss chard

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Introduction

- Bacterial leaf spot caused by *Pseudomonas syringae* pv. *aptata,* is a major threat to table beet (*Beta vulgaris* ssp. *vulgaris*) and Swiss chard (*B. vulgaris* ssp. *cicla*) production and causes yield losses worldwide.
- Within *P. syringae sensu stricto,* Phylogroup 2b (PG 2b), two genotypes (MLST1 and MLST3) appear to be the most aggressive (see poster P-020 and P-639).
- Seed transmission is a major avenue of dissemination, but available methods do not rapidly detect *P. syringae* pv. *aptata* strains present in seed lots or distinguish strains from non-pathogens.

Research Goal

- Our goal is to design a diagnostic assay to distinguish pathogenic *P. syringae* pv. *aptata* from nonpathogenic strains in plant and environmental samples.
- Here we report the development of a TaqMan qPCR assay for *P. syringae* pv. *aptata* MLST3.

Figure 1. Bacterial leaf spot symptoms. Table beet (top) and Swiss chard (below) (photo credit Lindsey du Toit).

Materials and Methods

Collection of Isolates

- Worldwide collection including 421 strains of *P. syringae* pv. *aptata*.
- Sources: infected beet and chard seeds, and leaf isolates.
- Both pathogenic and non-pathogenic strains were included.

Generation of SNPs phylogeny

- Collected genomic sequences of the target strains (phylogroup 2).
- Variant calling using parsnp tool performed to identify Single Nucleotide Polymorphisms (SNPs) across all genomes.
- Parsnp aligned the core genome of the input genomes, using *P. syringae* pv. *aptata* ICMP459^{PT} as the anchor.
- Maximal unique matches (MUMs) were identified to construct the multiple alignment, from which SNPs were extracted to generate a phylogenetic tree with IQ-TREE.

KEC Bioinformatics Pipeline

- Applied to whole-genome sequences of diverse *P. syringae* pv. *aptata* strains (including MLST1, MLST3, 2a, 2b, 2c, 2d, and non-pathogenic strains).
- Identified unique gene specific to MLST3.

Primer Design

- A specific region within the candidate MLST3 marker gene was selected.
- The marker gene was confirmed as unique to MLST3.

qPCR Assay Evaluation

- We evaluated 59 known MLST3 strains for amplification by the MLST3 protocol.
- 32 strains in MLST1 and 54 strains in phylogroups 2a, 2b (other than MLST1 and MLST3), 2c, 2d were also evaluated.

Results

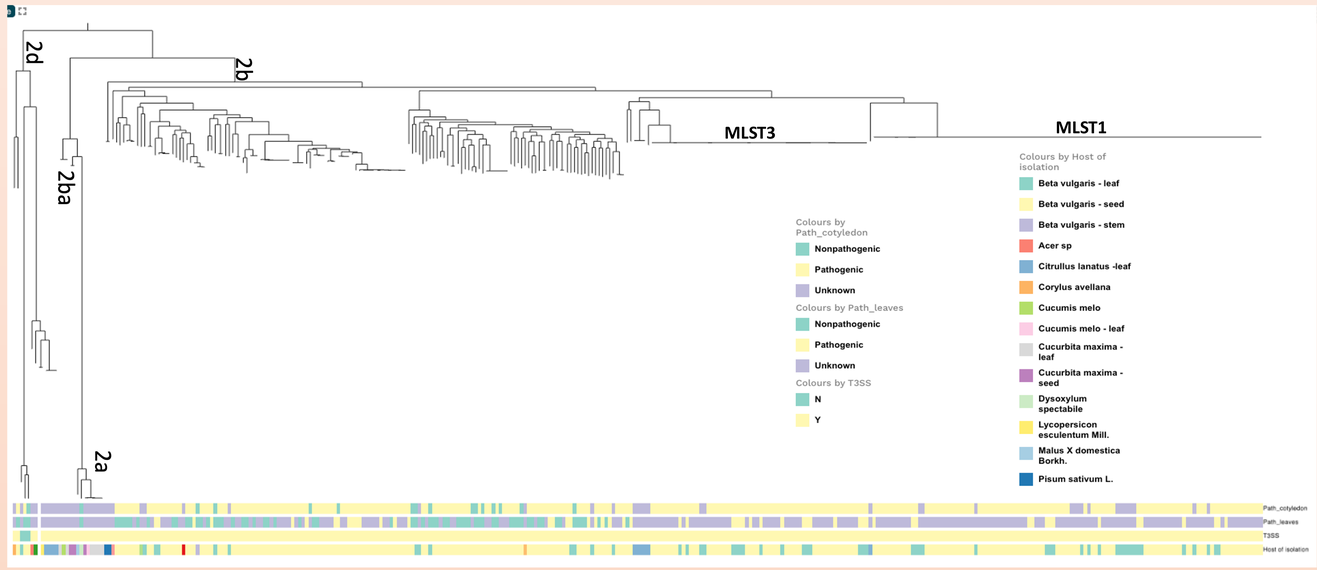

Figure 2. Phylogenetic analysis of *Pseudomonas syringae* pv. *aptata* strains in PG2, based on core SNPs. The analysis revealed 78 distinct genotypes based on phylogenetic analysis. Most aggressive strains clustered within MLST1 and MLST3 in phylogroup 2b (see poster P-010, P-020, and P-639).

Table 1. MLST 3 specific TaqMan qPCR amplification.

	MLST1	MLST3	PG 2a	PG 2b[a]	PG 2c	PG 2d
Number of strains evaluated	32	59	11	33	3	7
Number of strains amplified	0	59	0	0	0	0
Number of strains did not amplify	32	0	11	33	3	7

[a]PG 2 strains reported here did not include MLST1 or MLST3 strains.

Discussions and Conclusion

- We successfully identified a unique genetic marker specific to *P. syringae* pv. *aptata* MLST3 and designed a TaqMan assay.
- For the 145 strains tested to date there was 100% specificity.
- The assay offers a precise method for pathogen monitoring that should aid in early detection.
- Provides a valuable tool for targeted disease management in agricultural settings.

Future Directions

- We have used this assay to identify strains from seeds and are sequencing these strains for confirmation (see poster P-639).
- We are in the process of conducting assays with seed, plant material, and soil.

References

Derie *et al.,* 2016; *Phytopathology, 106*, Suppl 4, 142.

Pethybridge *et al.,* 2018; 10.3390/agronomy8070112.

Safni *et al.,* 2016. *Phytopathology, 106*, Suppl 4, 143.

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Seedborne cucurbits and chenopods diseases caused by *Pseudomonas syringae*

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