Genome-informed TaqMan qPCR assay precisely detects virulent *Pseudomonas syringae* pv. *aptata* genotype MLST1

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Introduction

- Bacterial leaf spot, caused by *Pseudomonas syringae* pv. *aptata*, is a significant threat to table beet (*Beta vulgaris* ssp. *vulgaris*) and Swiss chard (*Beta vulgaris* ssp. *cicla*) worldwide (1).
- Since the pathogen can be disseminated through contaminated seed, effective disease management relies on early, culture-independent screening to prevent outbreaks (2, 3).
- Within phylogroup 2b, we recently developed a highly specific TaqMan qPCR diagnostic for the aggressive MLST3 genotype (4). However, the equally virulent MLST1 genotype (5) still lacks a high-resolution detection tool.
- Traditional broad-spectrum diagnostics fail to differentiate MLST1 from closely related non-target genotypes including MLST3 (4).
- Comparative genomics identified unique genes on MLST1, enabling the development of a highly specific diagnostic assay.

Objectives

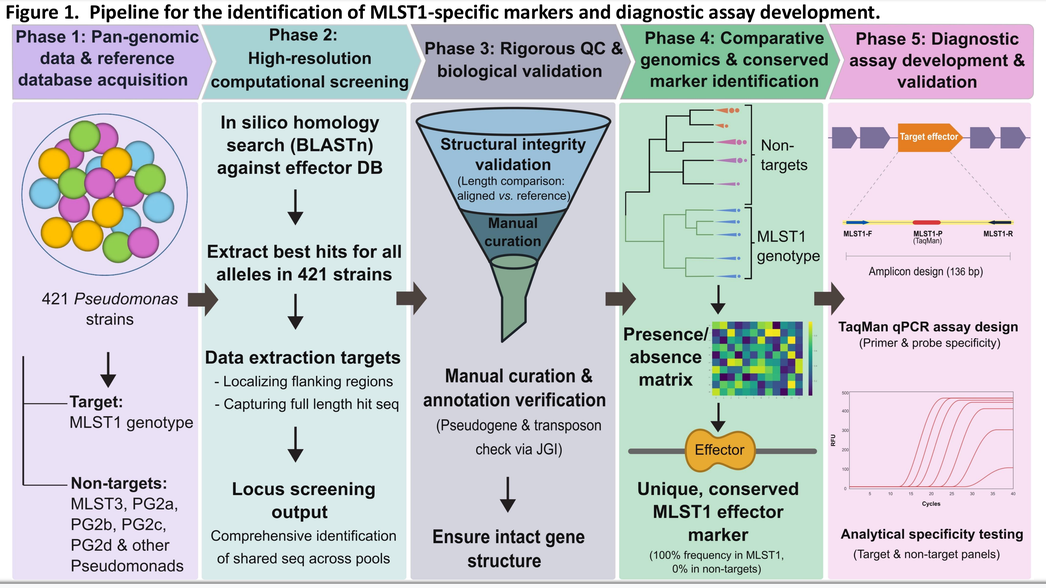
- Mine the pan-genomes of 421 *Pseudomonas* strains to identify conserved, unique effector loci specific to the MLST1 genotype.
- Develop and optimize a TaqMan qPCR assay targeting this unique effector sequence.
- Empirically validate the analytical specificity of the assay against a comprehensive panel of target and non-target phylogroups.

References

(1) Pethybridge *et al.,* 2018; 10.3390/agronomy8070112.
(2) Derie *et al.,* 2016; *Phytopathology*, *106*, Suppl 4, 142.
(3) Safni *et al.,* 2016. *Phytopathology, 106*, Suppl 4, 143.
(4) Hamidizade et al., 2026; *Plant Disease*, 10.1094/PDIS-03-26-0442-RE.
(5) Osabutey, S., et al. 2025; *Phytopathology*. Vol. 115. No. 12. 3340.

Materials and Methods

Figure 1. Pipeline for the identification of MLST1-specific markers and diagnostic assay development.



Results

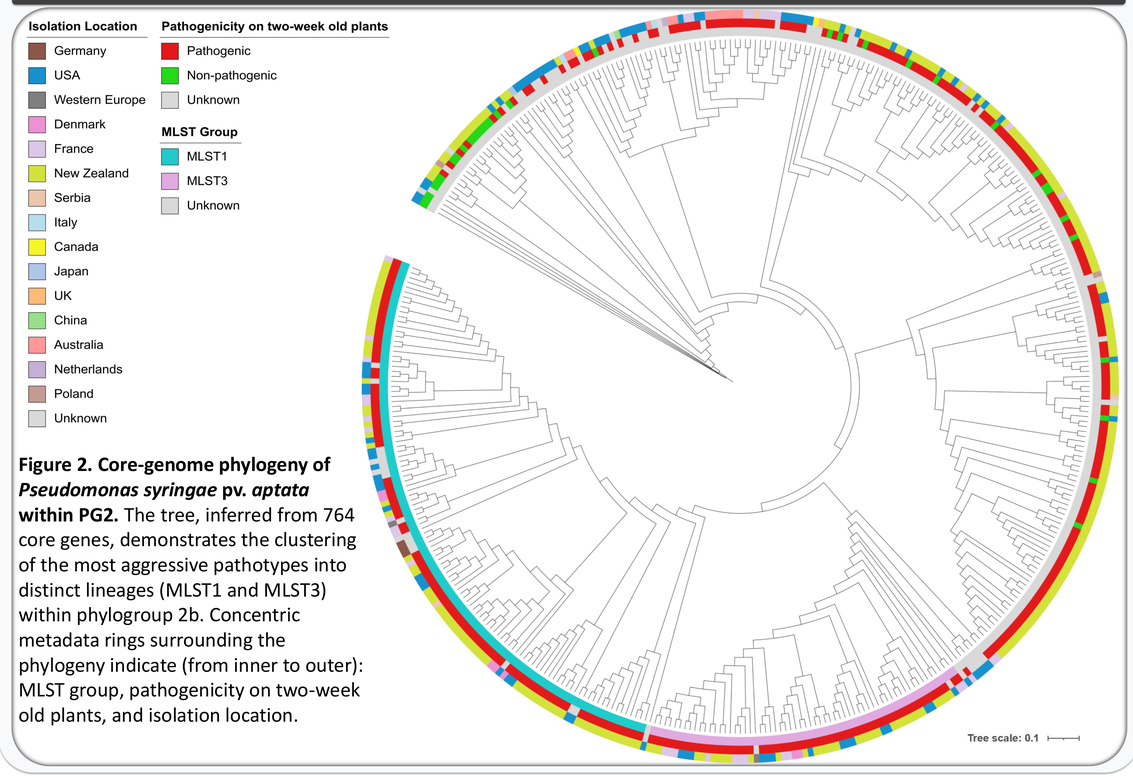


Figure 2. Core-genome phylogeny of *Pseudomonas syringae* pv. *aptata* within PG2. The tree, inferred from 764 core genes, demonstrates the clustering of the most aggressive pathotypes into distinct lineages (MLST1 and MLST3) within phylogroup 2b. Concentric metadata rings surrounding the phylogeny indicate (from inner to outer): MLST group, pathogenicity on two-week old plants, and isolation location.

Validation Outcomes and Conclusion

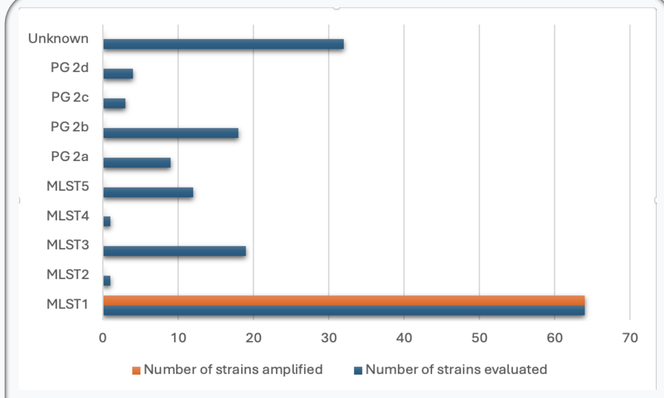


Figure 3. Analytical specificity of the MLST1 qPCR assay evaluated against 163 Phylogroup 2 strains. The assay exhibited 100% inclusivity for the target genotype (64/64 MLST1 strains) and absolute exclusivity, with zero cross-reactivity among the 99 non-target isolates.

- The assay demonstrated absolute exclusivity against closely related genotypes (including MLST3 and other PG2 subgroups), and inclusivity of MLST1 strains yielding 100% diagnostic specificity across the 163 evaluated strains.
- Ultimately, this effector-based framework provides a scalable blueprint for developing diagnostics against other emergent pathotypes, directly empowering proactive epidemiological surveillance and targeted disease management in *Beta vulgaris* production systems.

Future Directions

- Future studies will validate the assay directly on naturally-infected seeds and plant tissues to establish in-field diagnostic protocols.
- We aim to multiplex this assay with other lineage-specific markers to create a single-reaction diagnostic panel.

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