Diversity of seedborne *Pseudomonas syringae* pv. *aptata* isolates associated with bacterial leaf spot of table beet and Swiss chard

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Introduction

- *Pseudomonas syringae* pv. *aptata* is an economically important pathogen causing bacterial leaf spot (**BLS**) diseases on a wide variety of plants, including table beet and Swiss chard, globally.
- *Pseudomonas syringae* pv. *aptata* is a member of *P. syringae sensu stricto* (otherwise known as genomospecies 1 or phylogroup 2).
- Pathogens causing BLS on table beet and Swiss chard vegetable or seed crops are in subclade phylogroup 2b (Figs. 2 and 3), though other *Pseudomonas* species within the *P. syringae* subgroup also can cause BLS (Nampijja 2025).
- These pathogens can be seedborne and distributed long distance on seed.
- This poster presents preliminary data on diversity, pathogenicity, and fluorescence of strains isolated from beet and chard seed grown in France, New Zealand, and the USA.

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

Methods

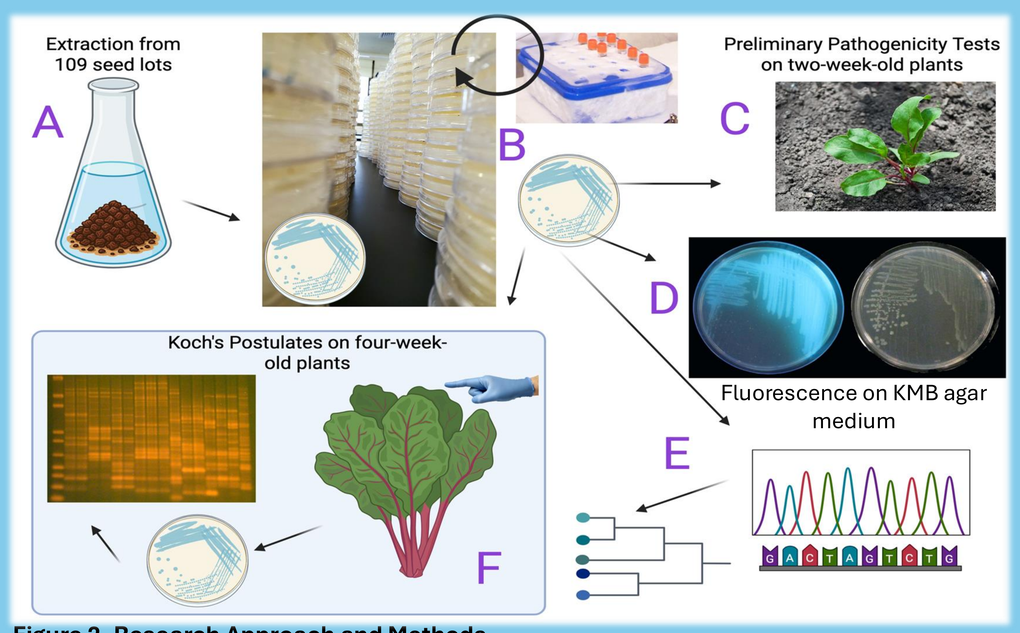

Figure 2. Research Approach and Methods.

A. Seed Sampling: Bacteria were extracted from seed grown in France, New Zealand, and the US from 2013-2019 using industry standard methods (Crane 2025). For each seed lot, 10,000 seeds were soaked in sterile saline solution for 4 h at room temperature, then placed on a shaker for 10 minutes at 150 RPM**.**

B. Supernatant of each was spread on KBC agar medium (Mohan and Schaad 1987), and single colonies streaked for purity and stored for further use.

C. Preliminary Pathogenicity Testing : 1,442 seed isolates were inoculated onto the adaxial and abaxial sides of true leaves of 2-week-old seedlings (three replicates) of the host from which they were isolated, by mechanical inoculation with a bacterial suspension of 0.5 OD. Leaf spot severity was evaluated 7 days after inoculation using a 1-5 rating scale that is categorized in this poster as high, mild, low, and non-pathogenic.

D. Fluorescence: Fluorescence was measured at 254 nm for strains on KMB agar medium.

E. Genotyping: Illumina whole genome sequencing was conducted by SeqCenter (Pittsburgh, PA). Genomes were prepared using published methods (Nampijja 2025), and a 951-core gene phylogeny made for 250 strains. A subset of strains was evaluated with Koch's postulates (Figs. 3 and 4, respectively). Alternatively, pathogens were identified using qPCR protocols specific for phylogroup 2 (Lacault et al. 2024), or MLST1 and MLST3 (Hamidizade et al. 2025 and unpublished).

F. Koch's postulates: Pathogenicity was evaluated on a subset of pathogens inoculated onto **4-week-old** seedlings of table beet ('Red Ace') and Swiss chard ('Silverado'). Disease severity was rated 14 days after inoculation. Percent disease was estimated using a 1 - 100% scale and strains were designated as causing high, mild, or low disease or as non-pathogenic based on those ratings. Reisolates from symptomatic tissue were used to confirm Koch's postulates by rep-PCR assay for all pathogenic isolates (not shown).

Results

Table 1. Pathogenicity tests and identity of *Pseudomonas* isolates from table beet and Swiss chard seed lots.

	Number of isolates				Pathogenicity test		Identity of pathogens		
	Seed lots	Isolates	Isolates sequenced	Isolates genotyped by PCR assay	2-week-old plants[a]	4-week-old plants[a]	PG2[b]	MLST 1[c]	MLST 3[b]
Beet	66	709							
France	0	0	0	0	0	0	0	0	0
New Zealand	22	477	210	0	440 (477)	10 (11)	ND[d]	50 (210)	20 (210)
United States	44	354	ND	ND	187 (354)	ND	ND	ND	ND
Swiss Chard	43	590							
France	5	59	5	36	42 (59)	ND	38 (59)	9 (59)	0 (59)
New Zealand	17	121	31	0	106 (121)	6 (15)	ND	1 (31)	1 (31)
United States	21	399	4	ND	257 (399)	ND	ND	ND	ND

[a] Number of strains pathogenic on the host of isolation (number of strains tested).
[b] Number of strains in phylogroup 2b or sequence type (number of strains tested).
[c] Preliminary data evaluated with endpoint PCR assay.
[d] ND = Not determined.

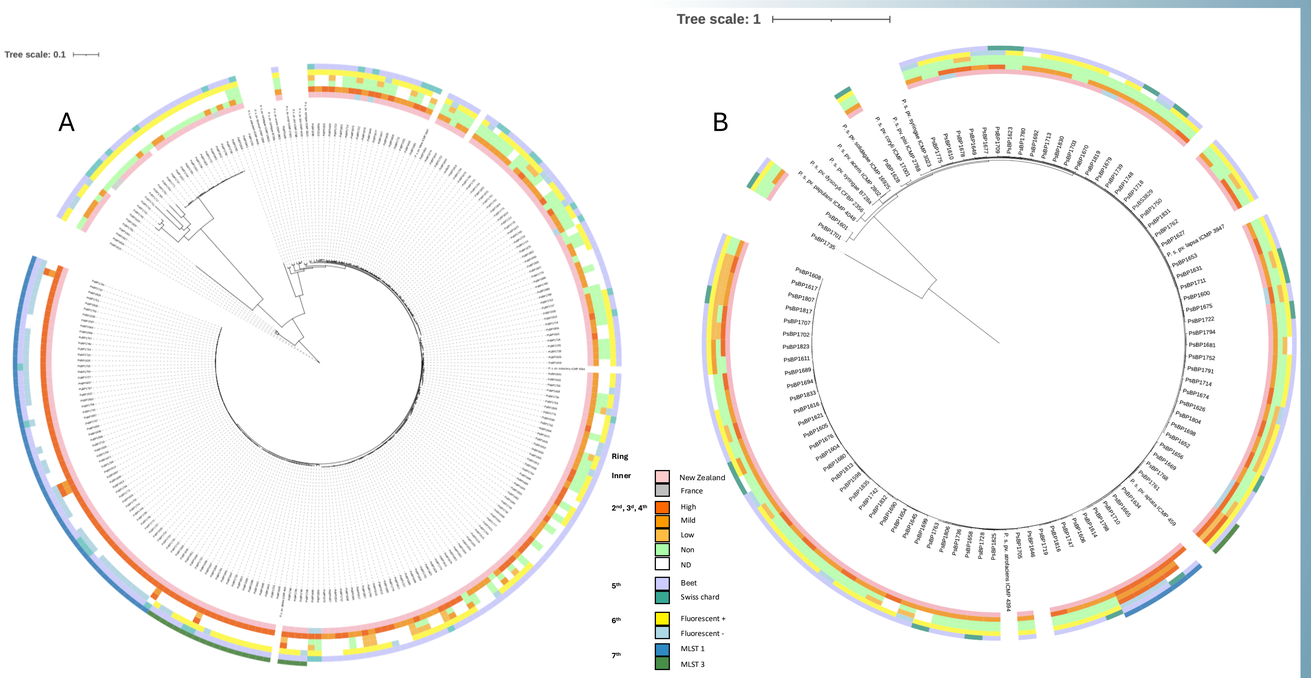

Figure 3. Phylogenic relationships of seed isolates. Phylograms compare isolates from seed to pathotypes in *P. syringae sensu stricto* (PG2) and a leaf isolate PsBS829 (PAP014) to: A) 259 seed isolates using 951 core genes, and B) a subset of 102 seed strains for which Koch's postulates were performed, using 4,254 core genes. The inner ring indicates the origin of the seed, rings 2, 3, and 4 indicate relative virulence of strains inoculated on 2-week-old seedlings of the host of origin, 4-week-old Swiss chard seedlings, and 4-week-old table beet seedlings, respectively. Rings 5, 6, and 7 indicate the host of origin, fluorescence on KMB agar medium, and designation of MLST from a previous 19 core gene phylogeny, respectively. The pathotype of *P. syringae* pv. *aptata* is a member of MLST3 (not shown).

Conclusions

- Bacterial leaf spot pathogens were isolated from table beet and Swiss chard seed lots grown in the USA, New Zealand, and France.
- Strains evaluated to date are found in *Pseudomonas syringae sensu stricto,* phylogroup 2b.
- Two sequence types, MLST 1 (non-fluorescent) and MLST 3 (fluorescent and identical to *P. syringae* pv. *aptata*), dominated the pathogenic strains.
- Other bacterial leaf spot pathogens found in phylogroup 2b were non-pathogenic or weakly virulent on 4-week-old seedlings.
- There were difference in virulence on 2- vs. 4-week-old seedlings, with 4-week-old seedlings showing immunity to strains that were weakly virulent on 2-week-old seedlings.
- Additional pathogenic isolates from France and the USA are being sequenced and evaluated to confirm their genetic identities.

Acknowledgements

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Citations

- Crane, S. A. 2023. Seed transmission of *Pseudmonas syringae* pv. *aptata*, and efficacy of bactericides for control of the pathogen in beet and Swiss chard seed production. Master's Thesis, Washington State University.
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