

# Comprehensive analysis reveals conserved prophages across *Pseudomonas syringae* species complex

NC STATE



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## Background

- The *Pseudomonas syringae* species complex (PSSC) includes over fifteen species and more than 60 pathovars, infecting a wide range of commercial crops worldwide, including cucurbits, beets, and chenopods (CBC).
- This project aimed to isolate and engineer phages with marker genes to enable pathogen detection in CBC.
- Although lysis zones were observed from many infected tissue lysates, none of these lysis zones were reproducible.

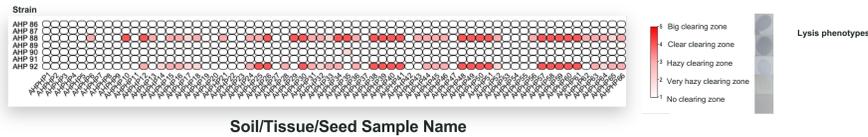


Figure 1: PSSC phage isolation attempts from 60 soil and tissue samples

**We hypothesized that the presence of prophages, prophage-like elements, and bacterial defense systems may be responsible for this resistance.**

## Objectives

- Benchmark prophage prediction tools (PHASTEST, PhiSpy, VIBRANT) using 15 publicly available PSSC type strains
- Predict prophages in 439 PSSC genomes sequenced in this project and characterize their diversity using Terminase large subunit gene and hierarchical protein clustering

## Methods

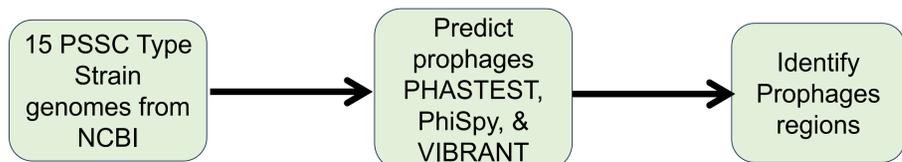


Figure 2: Optimization of phage prediction tools using 15 PSSC type strains

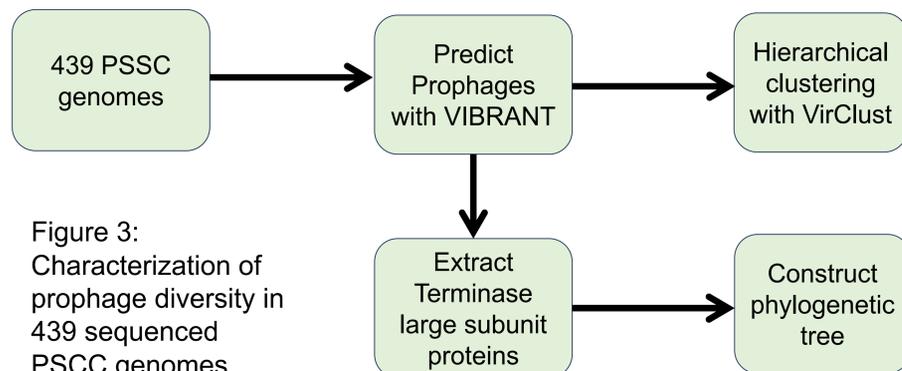


Figure 3: Characterization of prophage diversity in 439 sequenced PSSC genomes

## Conclusions

- 1421 prophages were identified across PSSC genomes.
- Prophages found in PSSC genomes contained all three types of packaging mechanisms.
- Hierarchical protein clustering showed presence of 65 conserved clusters in PSSC prophages, with one phage present in 398 genomes

## Results

### Summary of prophage prediction tools

A	<i>Pseudomonas</i> strains	PHASTEST	PhiSpy	VIBRANT
	<i>P. aeruginosa</i>	2	2	1
	<i>P. amygdali</i>	2	4	4
	<i>P. aptata</i>	5	-	-
	<i>P. cannabina</i>	-	-	2
	<i>P. caricapapayae</i>	-	13	10
	<i>P. coronafaciens</i>	3	3	2
	<i>P. viridiflava</i>	2	3	-
	<i>P. syringae</i> pv <i>aceris</i>	3	6	5
	<i>P. syringae</i> pv <i>aptata</i>	-	-	2
	<i>P. syringae</i> pv <i>atrofaciens</i>	3	5	3
	<i>P. syringae</i> pv <i>coryli</i>	5	1	5
	<i>P. syringae</i> pv <i>dysoxili</i>	2	4	3
	<i>P. syringae</i> pv <i>lapsa</i>	3	4	4
	<i>P. syringae</i> pv <i>papulans</i>	9	9	7
	<i>P. syringae</i> pv <i>pisi</i>	1	1	1
	<i>P. syringae</i> pv <i>syringae</i>	1	2	1

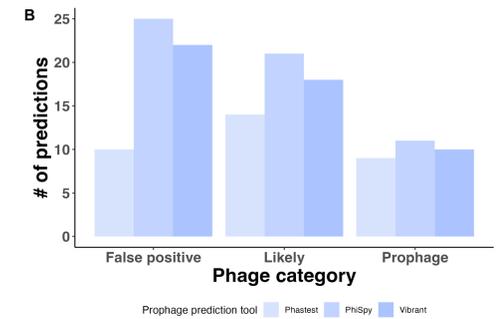


Figure 4: A) Total number of prophages identified by three prophage prediction tools. B) classification of predicted regions based on likelihood of being prophages

### Diversity of TerL proteins in PSSC



Figure 5: Phylogenetic tree of Terminase large subunit (TerL) proteins extracted from PSSC genomes and reference TerL sequences (colored branches)

### Hierarchical clustering of phages with VirClust

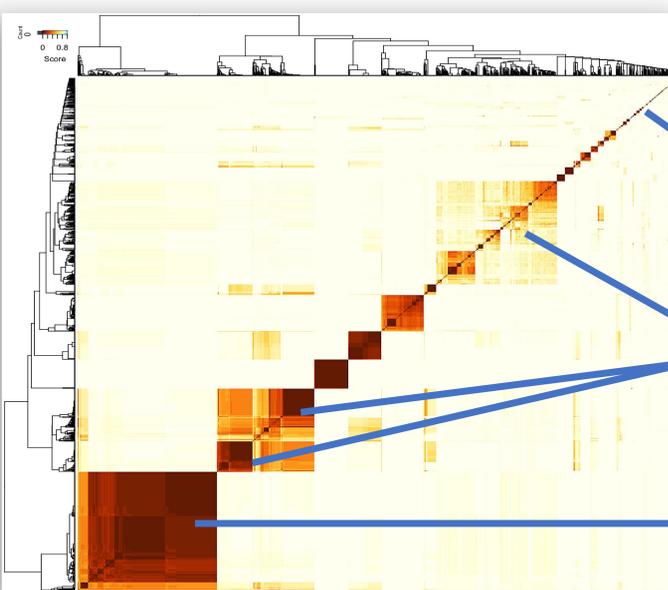


Figure 6: Hierarchical clustering of protein coding regions from 1421 predicted prophage regions extracted from PS genomes. 65 protein clusters were

Eleven Singletons with phages not sharing any phage proteins with other clusters

Phage clusters sharing a high number of proteins between them

Largest cluster with 400 prophage members conserved in 398 different PSSC bacterial genomes

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