

A BROAD-SCALE GENOMIC COMPARISON OF PSEUDOMONAS SYRINGAE STRAINS ASSOCIATED WITH CUCURBITS AND OTHER HOSTS

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Introduction:

Pseudomonas syringae is a ubiquitous and highly adaptable bacterial plant-pathogen. It is a member of the *Pseudomonas syringae* species complex (Pssc), which contains 15+ *Pseudomonas* species, including many plant pathogens, and is sub-divided into 13 phylogroups. Of these, Pg 2 contains the true members of the species *P. syringae* (sensu stricto).

P. syringae is the causal agent of many plant diseases, including bacterial leaf spot of cucurbits (BLS), which occurs worldwide, and periodically within the southeastern United States. Severe and widespread BLS outbreaks in the springs of 2013-14 in Florida prompted a survey to characterize bacterial strains associated with the disease in the region¹.

From these efforts, 2013-14 Florida isolates were identified solely as P. syringae strains

Goals:

Tree scale: 0.01

 Determine if cucurbit-associated *P. syringae* strains are closely related to strains isolated from other non-cucurbit plant hosts. From this we hope to infer whether BLS-associated *P.s.* strains may have the potential to infect other non-cucurbit hosts.
 Examine the level of genetic similarity shared between 2013-14 and 2020-21 Florida BLS-associated *P. syringae* strains

Figure 2. Maximum-likelihood phylogeny of all cucurbit-associated strains based on sequences of genes *rpoD*, *gltA*, *gapA*, and *gyrB*





belonging to Pssc phylogroups 2A and 2B¹. However, isolates collected during a later study conducted in 2020-21 included both members of Pssc phylogroups 2A and 2B, as well as additional diverse *Pseudomonas* species known to infect different plant hosts².

This unexpected diversity in pseudomonads associated with BLS prompted further examination of cucurbit-associated strains of *P. syringae*, the traditional causal agent of the disease. In this study, we use many bioinformatic tools, as well as all *P. syringae* genomes available from NCBI, to conduct genome-wide comparisons of cucurbit and non-cucurbit-associated strains.

Figure 1. K-means cluster analysis of 840 genomes previously identified as P. syringae





Discussion:

Publicly Available Data:

- Numerous genomes submitted to NCBI as P. syringae are actually other *Pseudomonas* species according to ANI, isDDH, and TYGS results. Care should be taken when using such publicly available data in the future
- The scope of this study is limited to published data, which favors *Pseudomonas* strains associated with plant disease and economically significant crops, and does not accurately represent the diversity of *Pseudomonas syringae* strains present in the environment

K-means Clustering and Identification of Unknown Strains:

- Members of *P. cerasi* and *P. syringae* are not always distinguishable using ANI analysis, suggesting the potential existence of a species continuum, rather than two distinct species
- Identification of Pg 2A strains as *P. cerasi* and identification of many members of Pg 2D as an undescribed species of *Pseudomonas* may necessitate the re-examination of which Pssc phylogroups comprise *P. syringae* sensu stricto
- Cucurbit-associated Pg 2A strains clustered with other strains isolated from diverse hosts, implying that they may also possess the ability to infect non-cucurbit hosts
- The majority of cucurbit-associated Pg 2B strains formed a distinct cluster separate from strains isolated from other hosts, implying host specificity to cucurbits

Further Analysis of Cucurbit-Associated Strains:

• The majority of cucurbit-associated strains collected worldwide were placed into Pg 2B, supporting the hypothesis of **2B strains as cucurbit-specific pathogens and 2A**

Stars signify the presence and number of cucurbit-associated strains within a cluster Axis labels designate the amount of variation explained by each axis

Results:

Comparison to Type Strain:

- Only 251 of the 840 examined genomes were within ≥95% ANI range to the *P. syringae* type strain to belong to the species
- Cucurbit-associated strains within Pg 2A were identified as members of *Pseudomonas cerasi* on the basis of ANI, isDDH, and comparison to the TYGS
- Many cucurbit-associated strains within Pg 2B were within ≥95% ANI to both *P. syringae* and *P. cerasi*, and identified only as *P. syringae* using isDDH

K-means Clustering and Identification of Unknown Strains:

- All Florida isolates and the majority of cucurbit-associated strains from around the world were placed witthin three clusters, which corresponded to Pssc phylogroups 2A and 2B
- Cucurbit-associated strains within Pg 2A clustered with other strains isolated from diverse plant hosts
- Nearly all cucurbit-associated strains within Pg 2B formed a distinct cluster separate from strains isolated from other plant hosts
- Other clusters were identified to contain multiple *Pseudomonas* species, including *P. avellanae*, *P. amygdali*, as well as potential new species

Further Analysis of Cucurbit-Associated Strains:

- In MLSA phylogeny, the majority of cucurbit-associated strains collected in Florida and worldwide were placed into Pssc phylogroups 2A and 2B, with a few placed into 2D
- Pg 2B contains the most cucurbit-associated strains (n = 51)
- In both phylogroups 2A and 2B, in core-genome phylogenies, Florida

P121 2013, Chile, Squash P118 2013, France, Squash P12832 2006, France, Squash BRIP65019 a 2016, Zucchini, Australia P113 2011, Thailand, Squash BRIP65014 d 2016, Zucchini, Australia ZUM3584 Unknown, Italy, Squash PS711 2013, Serbia, Squash K9 2020, Florida, Watermelon BRIP65014 b 2016, Zucchini, Australia 13 C2 2013, Florida, Watermelon BRIP64883 a 2016, Zucchini, Australia P22 2009, USA, Squash

Large bolded numbers represent Pssc phylogroups Bootstrap values based on 100 replicates indicated at branching points

Figure 4. Maximum-likelihood phylogeny of Pssc phylogroup 2B cucurbit-associated strains based on core-genome analysis



members as generalist plant pathogens

According to core-genome phylogenies, Florida cucurbit-associated strains
collected in different time periods (2013-14 vs 2020-21) are more closely related to
strains from other countries than to each other. This could possibly be explained by
the seedborne nature of BLS-associated *P. syringae* strains and centralization of
cucurbit seed production within a few regions worldwide. This could potentially facilitate
the spread of a single, or multiple genetically similar, *P. syringae* strains from one seed
production location to many areas worldwide

Figure 3. Maximum-likelihood phylogeny of Pssc phylogroup 2A cucurbit-associated strains based on core-genome analysis



Bootstrap values based on 100 replicates indicated at branching points

Citations:

1. Newberry, E. A., Babu, B., Roberts, P. D., Dufault, N. S., Goss, E. M., Jones, J. B., & Paret, M. L. (2018). Molecular epidemiology of *Pseudomonas*

cucurbit-associated strains collected in different time periods (2013-14 and 2020-21) grouped more closely with strains collected from other countries than to each other
ANI between Pg 2A 2013-14 and 2020-21 strains ranges from 98.81% - 98.92%
ANI between Pg 2B 2013-14 and 2020-21 strains ranges from 99.06% - 99.09%

Bootstrap values based on 100 replicates indicated at branching points

syringae pv. *syringae* causing bacterial leaf spot of watermelon and squash in Florida. Plant Disease, 102(3), 511–518.

2. Fullem, K. R., Pena, M. M., Potnis, N., Goss, E. M., Minsavage, G. V, Iriarte, F. B., Holland, A., Jones, J. B., & Paret, M. L. (2024). Unexpected diversity of pseudomonads associated with bacterial leaf spot of cucurbits in the southeastern United States. Plant Disease, 108(3), 592–598.

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