Using *Psa* to understand how pathogens evolve during host infection

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Context

Do plant pathogens require all their effectors to cause disease - or just a few?

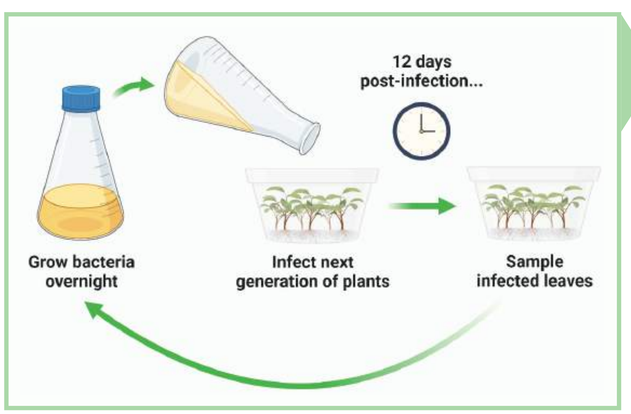
- Effector proteins help specialised pathogens invade the host, extract nutrients and suppress host immunity[1].
- *Pseudomonas syringae* pv. *actinidiae* (*Psa*) is an emergent kiwifruit pathogen that has a variety of potentially redundant effectors.
- Currently, only a few of *Psa*'s effectors appear to be essential for bacterial growth within the host plant[2].
- Kiwiberry (*A. arguta*) has strong *Psa* resistance because it can recognise several *Psa* effectors[3].

Why, then, does *Psa* retain nearly thirty functional effectors? Do these "non-essential" effectors make some unknown contribution to pathogenicity? To find out, we need a more sensitive way to detect contributions to pathogenicity.

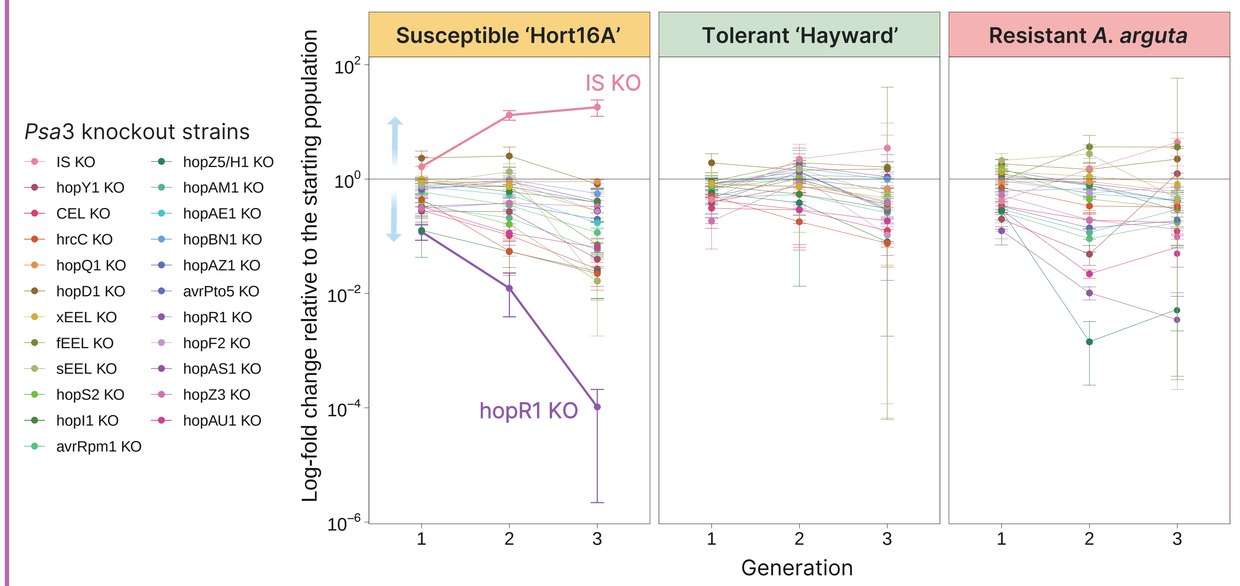
Which effectors are required to cause disease on different kiwifruit species?

Is there redundancy in the *Psa* effector repertoire?

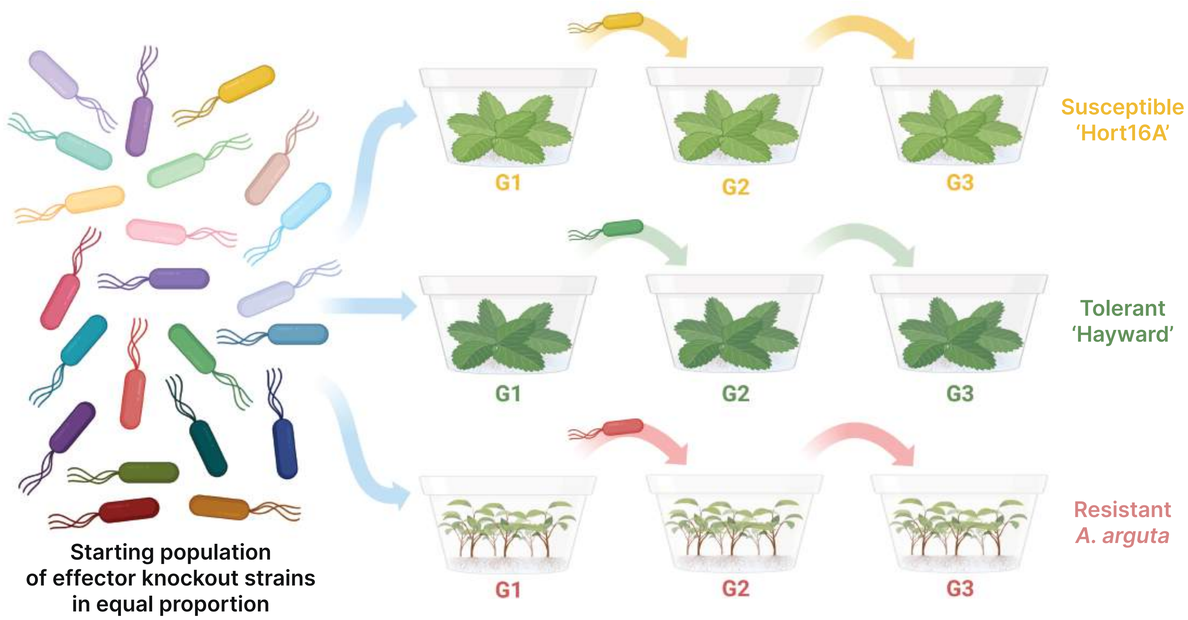
Does host selection refine the effector repertoire over time?



quantitative PCR was used to **track the presence of each knockout strain in the population** over time



Serial passaging allows us to more sensitively detect subtle contributions of effectors to virulence due to the **selective pressure exerted by competition** and the **narrow bottlenecks produced by passaging**



Results

The winner takes it all...

The *Psa*3 *IS* knockout strain is a control with *Psa*'s complete set of effectors, which outcompeted all of the other strains and took over the population in *Psa*-susceptible 'Hort16A'. While these effectors previously appeared to be individually redundant, they may be **collectively required** for successful pathogenicity.

... the loser has to fall

Several effector knockout strains significantly decreased in the population over time. Notably, the *Psa*3 *hopR1* knockout strain dropped out of the population entirely in 'Hort16A'.

Of the four *Psa* effectors recognised by *Psa*-resistant *A. arguta*, only the EEL knockout strains lacking *hopAW1* appeared to fight it out for the top spot. The other recognised effector knockout strains decreased in the population, despite their ability to escape recognition.

This research provides important insights into the genetic evolution of emergent pathogens.

Plant host resistance genes recognise the action of specific pathogen effectors. By understanding effector requirements and redundancy, we can identify which resistance genes are durable breeding targets.

This will ultimately inform robust resistance breeding efforts to sustainably manage pathogen outbreaks and reduce the need for chemical applications.

Acknowledgements

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1. **Xin et al. 2018.** Nature Reviews Microbiology 16:316.
2. **Jayaraman et al. 2020.** Molecular Plant Pathology 21:1467-1480.
3. **Hemara et al. 2022.** PLoS Pathogens 18:e1010542.