

From Soil to Solution: Isolating Bacteriophages from the Environment to Combat American Foulbrood





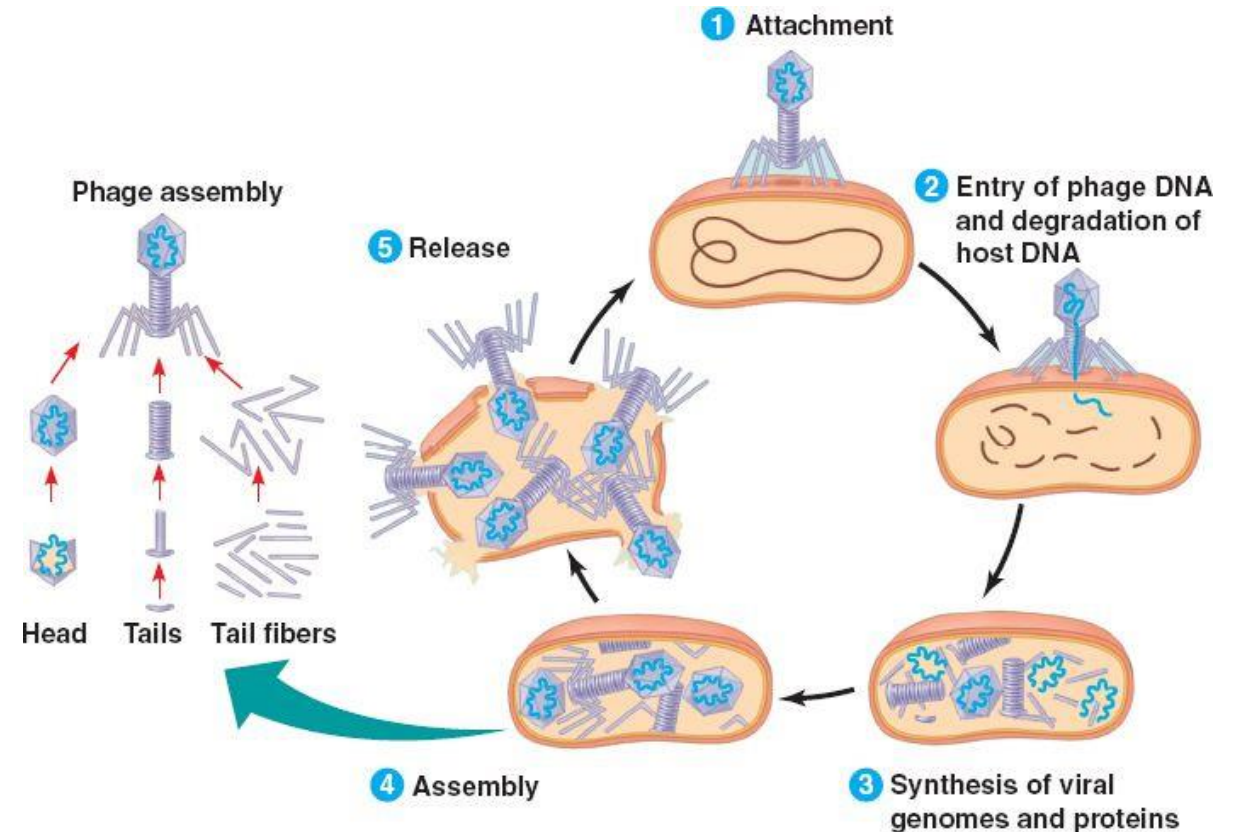
American Foulbrood (AFB)

- Most serious disease that infects honey bees
- Caused by bacterium *Paenibacillus larvae*
- Infected by spore form
- Infected hives must be destroyed immediately



Bacteriophages - What are they?

- First discovered in the early 1900's
- Combats bacteria in application known as phage therapy
- Self-propagating viruses that only infect bacteria
- Estimated 10^{31} globally





Phages - How are they used?

- Used for bacterial crop diseases
- FDA given 'generally recognized as safe' (GRAS) status to 10 phage cocktails
- Erwiphage used in Hungary for fire blight
- Biolyse used in UK for soft rot
- Have also been used against human bacterial infections



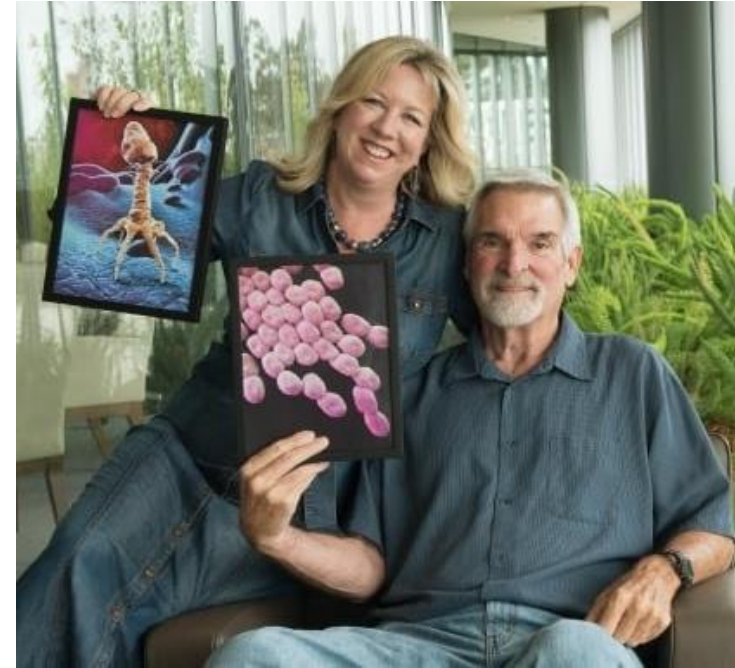
Phages - The Perfect Predator

- Vacationing in Egypt when hit with stomach bug
- Most dangerous, antibiotic-resistant bacteria in the world
- His wife convinced doctors to give bacteriophages a try



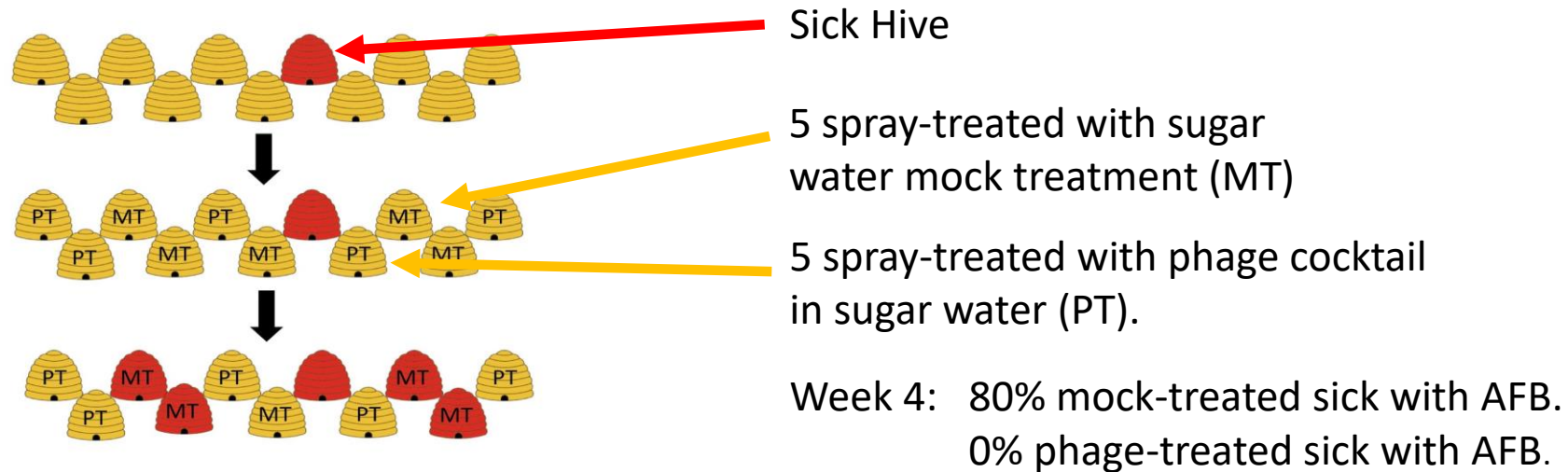
Phages - The Perfect Predator

- Tom came out of his coma and made a full recovery



Back to Phages for AFB

- Work undertaken at Brigham Young University, Utah
- 10 healthy hives used to determine phages ability to protect against infection when housed near a sick hive



Phage Cocktail Approach

- Work undertaken at the University of Nevada Las Vegas
- Created phage cocktail to administer to larvae
- A cocktail of phages can combat resistance

	<i>Paenibacillus larvae</i>										
Known ERIC Groups	I							IV		III/IV	IV
Isolated Phages	NRRL B-2605	2188 Isolate	NRRL B-3554	ATCC25748	ATCC25747	NRRL B-3650	2231 Isolate	ATCC 25367	ATCC49843	ATCC3688	ATCC25368
Halcyone											
Willow											
Harrison											
Fem											
Vadim											
Hermione											
Hayley											
Erin											
Heath											
Diane											
Charlie											
Bella											
Vegas											
Paisley											
Scottie											
Alexis											
Iowa											
Valery											
Summerlin											
Beta											
Hope											
Carly											
Xenia											
Holly											

Yost, D. G. et al.





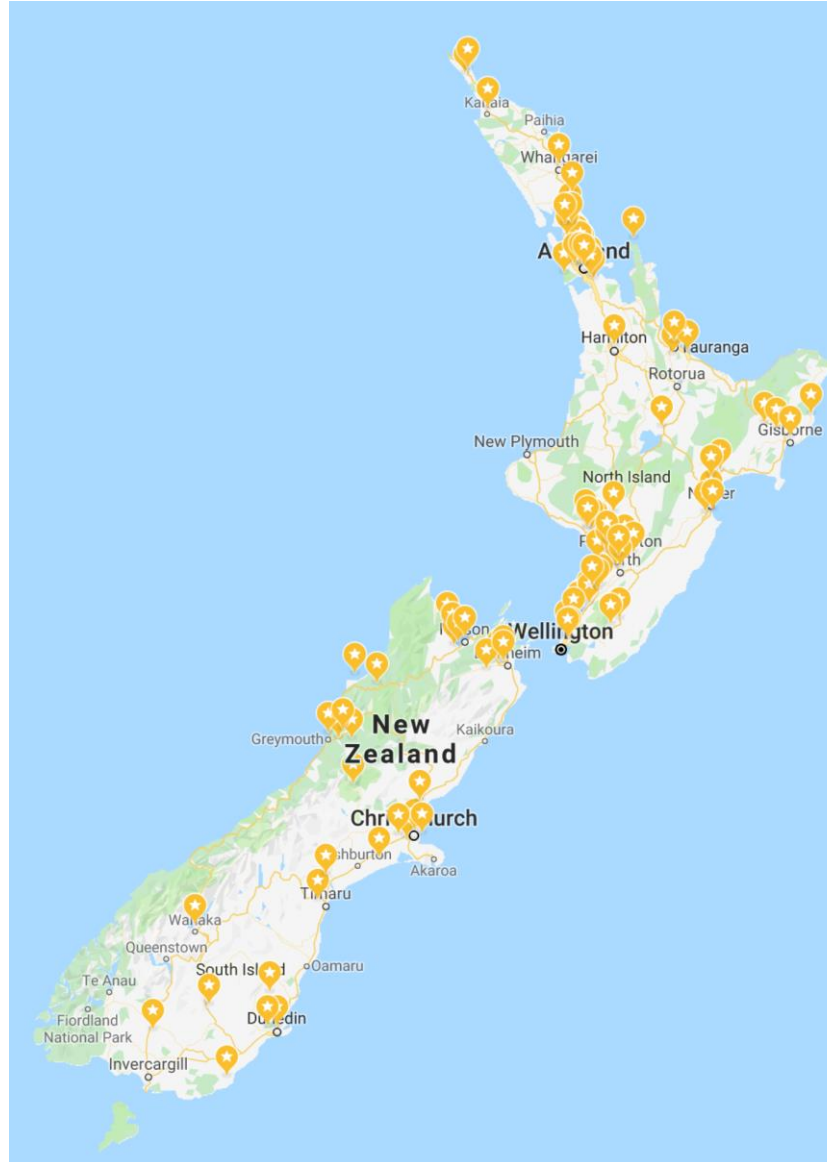
Paenibacillus larvae Strains in NZ

- Previously studied collections of *P. larvae* destroyed
- AsureQuality sending suspect brood frames
- Isolated eight positive *P. larvae* strains



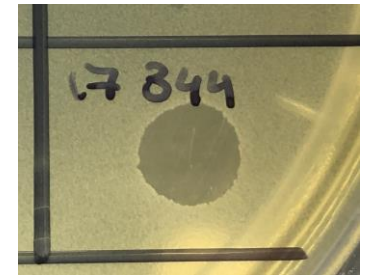
Phage Hunt

- 395 soil samples processed so far
- Locations from around NZ



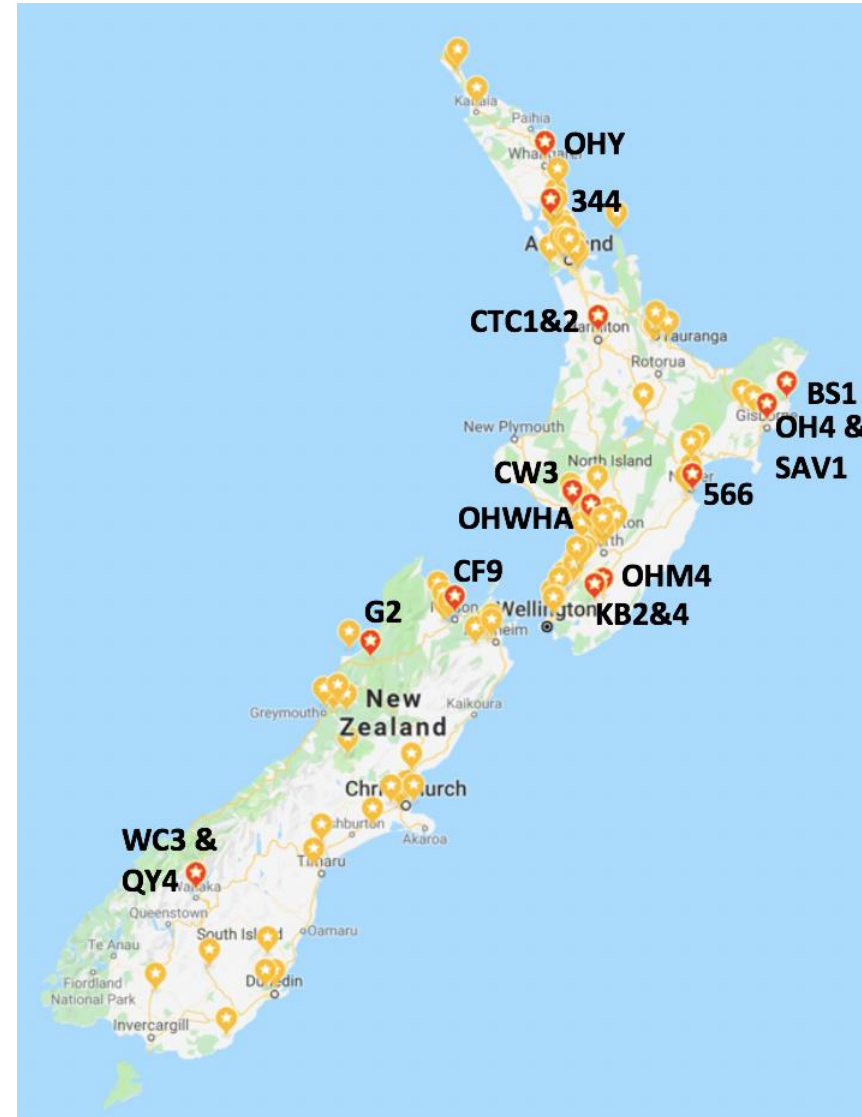
How do we look for Phages

- Collect soil/bee debris
- Flood this with solution
- Filter for only the tiniest particles (smaller than bacteria)
- Put on *P. larvae* to look for death

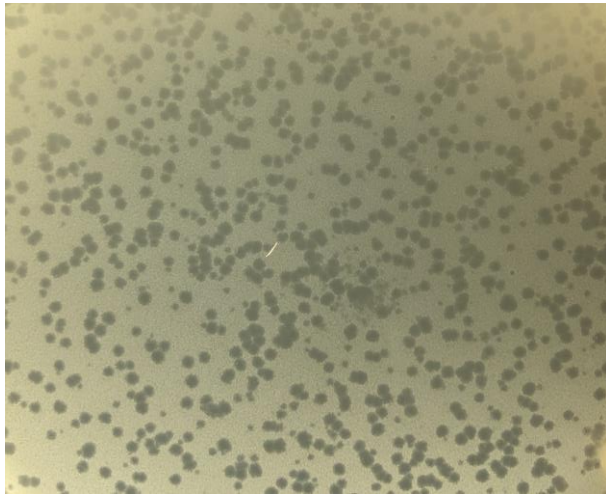


Phages Found so Far

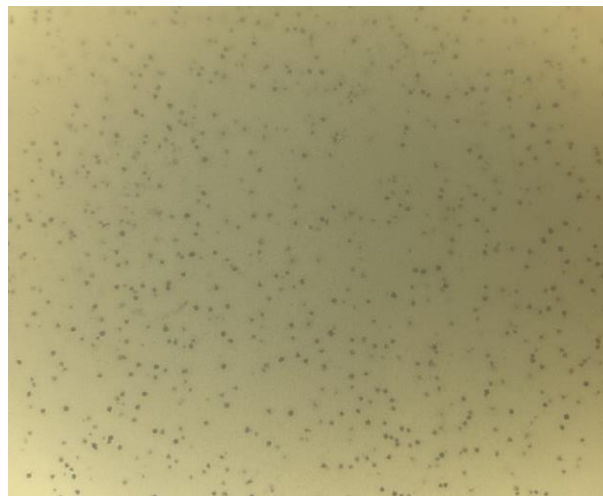
- 17 positive soil samples
- Positives from around NZ
- Collected from healthy hives without AFB infection



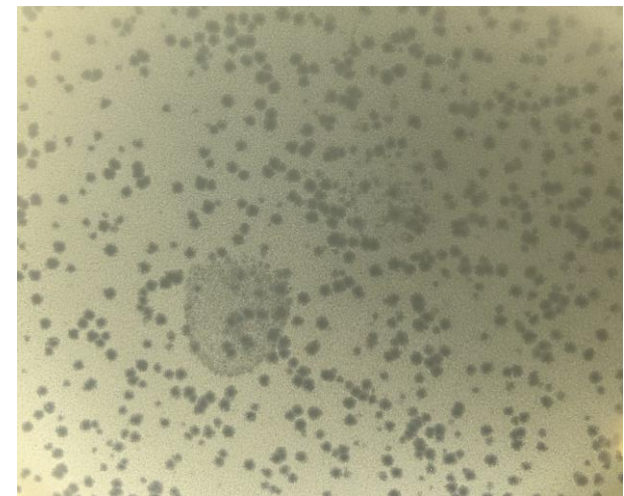
Phage killing *P. larvae*



**Phage 566
Isolate 2017**



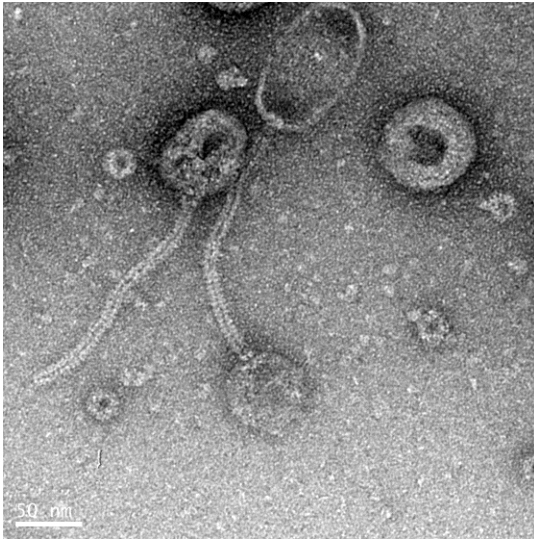
**Phage KB2
Isolate F1A**



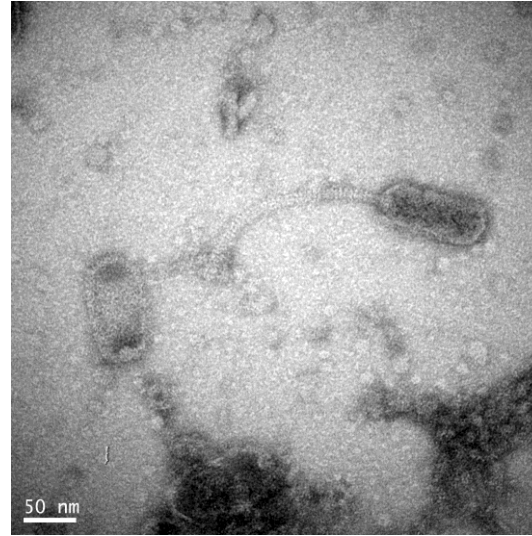
**Phage CTC1
Isolate TP**



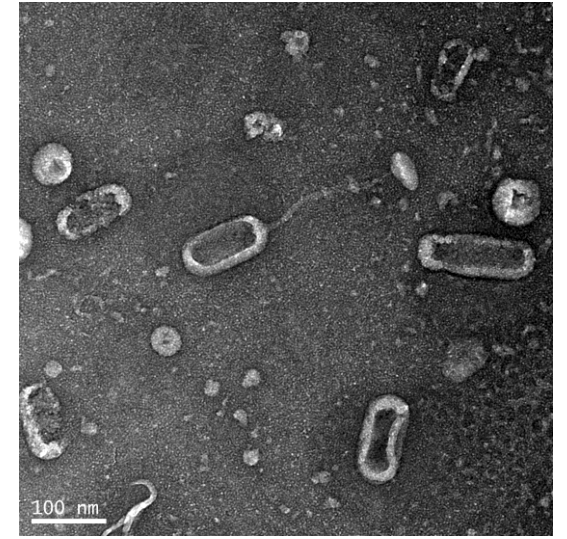
Phages Under Electron Microscope



Phage BS1



Phage OHWHA

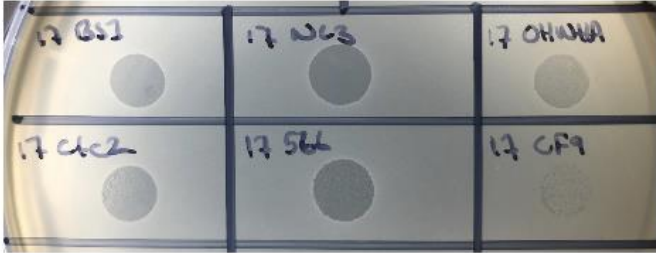
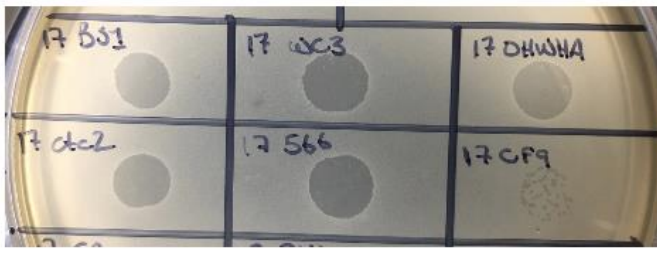


Phage WC3



Host Range of Phages

- Two distinct host infection patterns
- Some *P. larvae* isolates unable to be infected

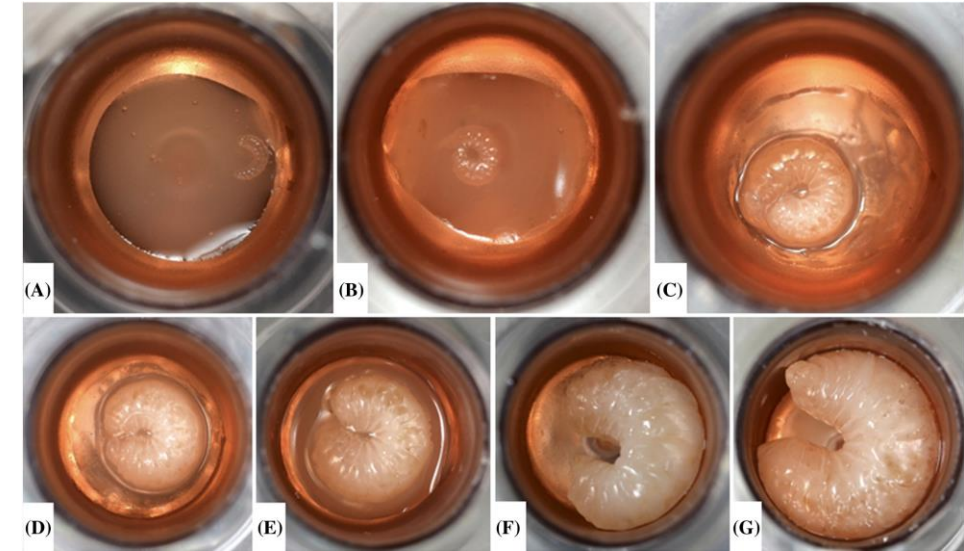
Isolate PI-F1A	Isolate PI-PaFR-2017
	

NZ Bacterial Isolates									
N Z B a c t e r i o p h a g e s		F1A	F2B	WAI	2017	TP	CHCH	2006	P1627
	WC3								
	QY4								
	CF9								
	G2								
	344								
	OHWH								
	OH4								
	CTC1								
	CTC2								
	BS1								
	566								
	SAV1								
	OHM4								
	KB2								
	OHY								



Future Directions

- Sequence phages
- Test phages on as many strains of *P. larvae* as possible
- Produce a safe bacteriophage cocktail of robust *P. larvae* phages
- Infect bee larvae with phage 'cocktail' in 24 well plates



From grafting (A) to 6 days post-grafting (G)



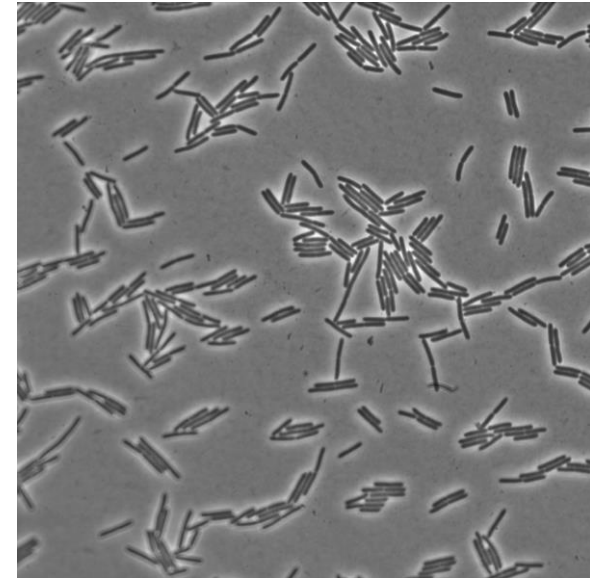


Complementary Hosts

- Non-pathogenic
- Related to *P. larvae*
- Phage for related hosts may infect *P. larvae*

Work so far

- 5 complementary hosts
- *Paenibacillus* Ash et al. 1994 (NZ)
- 80 samples processed, no phage yet 😞



How can you Help!

- Come see me at my poster to collect your sample pack



BEES ARE IMPORTANT TO NEW ZEALAND

American FoulBrood (AFB) is caused by a bacterium that infects Honeybees.

Bacteriophages are viruses that can kill bacteria.

Bacteriophages can be found ANYWHERE

Bacteriophages are the most numerous entity on the planet and there are bacteriophages in our soil that can kill the AFB bacterium.

Bacteriophages have two life cycles, lytic and lysogenic. The lytic bacteriophages are fast and effective killers and in the USA these have been shown to be able to protect Beehives from becoming sick with American FoulBrood disease.

Send us soil to help us find bacteriophages to kill AFB!

At Massey University, we are working to discover the bacteriophages that will kill the American FoulBrood causing bacteria. These will be completely sequenced and tested for safety. This work provides the groundwork for an innovative approach to naturally protecting NZ beehives.

Visit our website: Abate.massey.ac.nz to learn how you can help out!

ABATE
Active Bacteriophages for AFB Evaluation



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