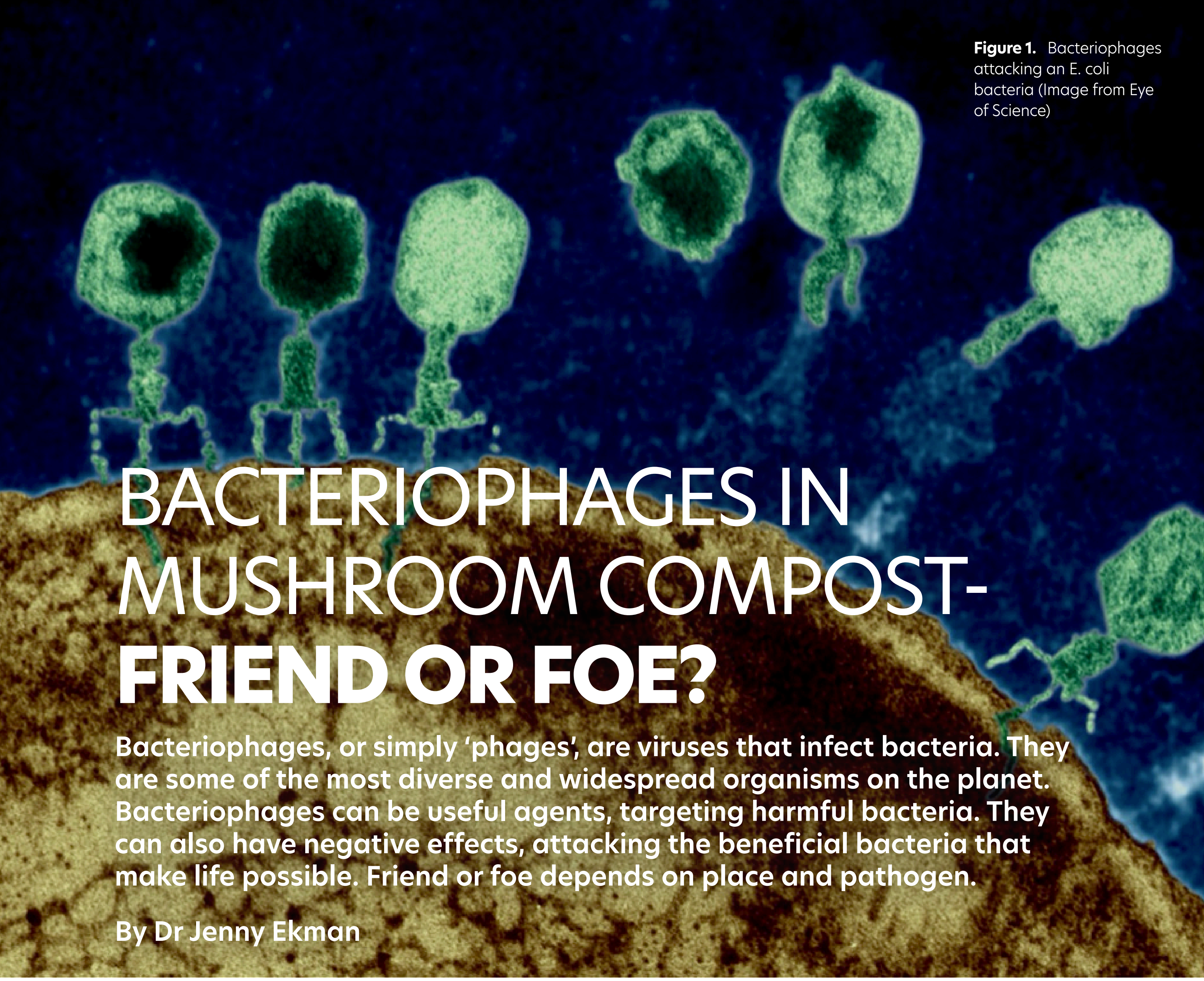


Figure 1. Bacteriophages attacking an E. coli bacteria (Image from Eye of Science)

BACTERIOPHAGES IN MUSHROOM COMPOST- **FRIEND OR FOE?**

Bacteriophages, or simply 'phages', are viruses that infect bacteria. They are some of the most diverse and widespread organisms on the planet. Bacteriophages can be useful agents, targeting harmful bacteria. They can also have negative effects, attacking the beneficial bacteria that make life possible. Friend or foe depends on place and pathogen.

By Dr Jenny Ekman

Bacteriophages as medicine

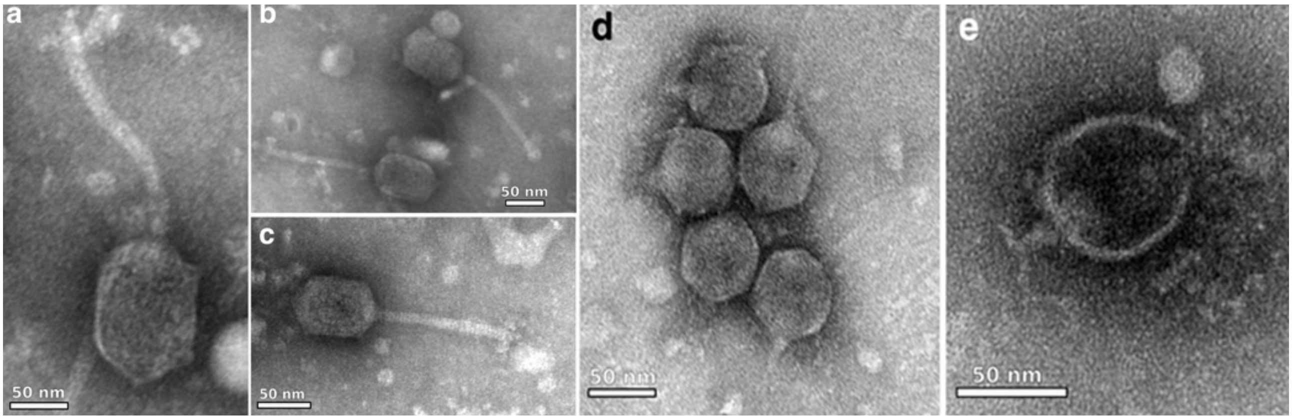
Bacterial diseases are notoriously hard to control. Inevitably, mushroom growers will be familiar with *Pseudomonas tolaasii*, the cause of brown blotch. Infection can occur through the substrate or from contaminated equipment. Control is challenging as there are no chemical controls and the bacteria can persist indefinitely in the damp conditions inside mushroom farms.

Using bacteriophages to control bacteria has become a popular research topic, which may take on some urgency with the rise of antibiotic resistant bacteria. However, bacteriophages have been in an arms race with bacteria since life on the planet was nothing but single celled organisms and algae. Ongoing and rapid evolution means that bacteriophages are frequently specific, not only to a bacterial species, but also to a particular variant.

For example, a recent (2022) study isolated 42 bacteriophages active against 23 different strains of brown blotch. Some of the phages could attack two or more brown blotch strains, whereas others were specific to a single strain.

The researchers therefore created a cocktail of eight different phages. This cocktail was sprayed onto an infected mushroom crop, completely preventing the development of symptoms of brown blotch during both first and second flush[1].

Unfortunately, the reproductive speed and genetic elasticity of bacteria means that such successes are often short lived.



Electron microscope images of phages that attack Pseudomonas bacteria. a-c are from the Siphoviridae family, d and e are from the (short tailed) Podoviridae family. Images from Amgarten et al., 2017.

Bacteriophages as farm workers

Many bacteria are not only 'not bad', but essential to composting. However, the range of bacterial species present is far from constant. In fact, great bacterial communities rise and fall during the composting process. For *example*, the *Acinetobacter* in phase 1 are succeeded by *Bacillus*, then uncharacterised proteobacteria and finally *Thermus*. Phase 2 sees the rise of *Pseudoxanthomonas* and *Steroidobacter*, which are joined during Phase 3 by *Mycovorax*[2].

And the role of bacteriophages in this process? Nobody knows….

Enter Rebecca Martin, a University of Sydney student with a major in microbiology and a love of fungi. Working with Professor Michael Kertesz, Ms Martin soon became fascinated by the mysterious world of bacteriophages.

Rebecca Martin, PhD candidate, sampling compost to test for bacteriophages.

"I've long been interested in fungi but I wanted to do something different for my honours research. No-one had looked for bacteriophages in *Agaricus* mushroom compost before," commented Ms Martin.

"We know that phages play vital roles in nutrient cycling (by killing bacteria they release nutrients) as well as in bacterial evolution. However, much of the work on phages has focussed on those found in marine environments, with only limited research on the terrestrial environment, partly because it is so difficult to extract and study these phages."

Phages drive bacterial evolution, potentially controlling what lives or dies. Whereas temperate phages simply use the genetic machinery of their host to survive, virulent phages kill their host, hunting them down like tiny ants attacking a much larger grasshopper.

"The increases that occur in certain bacterial species during composting, rapidly followed by population crashes, strongly resembles the 'kill the winner' pattern found for other bacteria:phage interactions," suggests Ms Martin.

"It's possible that phages are playing an essential role in nutrient cycling during the composting process".

Finding phages

Phages have been successfully isolated from a range of different compost samples using two different techniques.

The first involves growing a layer of bacteria-on an agar plate, called a bacterial 'lawn'. An extract potentially containing phages is added. If bacteriophages are present, they create a 'plaque', a hole in the bacterial layer where the bacterial population has been destroyed. The number of plaques can indicate the relative density of the phage population.

The second is a molecular approach. This is limited by the lack of a single, universal gene common to all phages. However, methods are available to detect T4 type phages based on the presence of the 'g23' gene. The T4 group includes the majority of phages isolated (so far) from terrestrial environments.

During her honours project Ms Martin used a combination of these techniques to identify phages in mushroom compost, as follows:

- Bacterial strains isolated from compost (as well as other sources) were grown on agar plates
- Phages were identified through formation of plaques in these bacterial lawns
- These were then isolated and grown to obtain pure phage cultures
- DNA was extracted for whole genome sequencing

As an additional activity, Ms Martin took samples at four different time points during the composting process at four different yards. PCR techniques were then used to identify T4-type phages present and compare populations.

Bacteriophages are common in mushroom compost, however their impacts on yield and quality are completely unknown

Phages found - *the story so far....*

Diverse populations of phages were found in both Phase 1 and phase 2 compost. These included both T4 types and the *Pseudomonas* infecting Yuavirus phage subfamily. This second group was abundant during both Phase 1 and Phase 2 composting. At least one of these phages was especially lethal to the *P. aeruginosa* bacterial strain used for isolation, while others appeared likely to have an extended host range. They were highly temperature tolerant, with activity not significantly reduced at temperatures up to 70°C.

Interestingly, diverse communities of T4 phages were also found at each of the four compost yards during both Phase 1 and 2 composting. However, each yard had its own, distinct phage population.

"These results are really a tantalising glimpse into the previously unknown world of bacteriophages in mushroom compost," comments Ms Martin.

"The results could help us better understand the overall microbial community. Interactions between bacteriophages and their microbial hosts could affect not only the composting process, but also the subsequent growth of mushrooms".

These results now form the foundation of Ms Martin's PhD "Exploring the bacterial phage community in mushroom compost". Specifically, Ms Martin hopes to determine whether the *Pseudomonas* phages can infect the *Pseudomonas* species that are present in casing. Throughout the project, there is also scope to examine which phages are present in casing and how the presence (or not) of phages affects mushroom yield and quality.

As we delve ever deeper into the world of the unimaginably tiny, it seems just possible the next composting era will be the Age of the Phage.

Hort Innovation Strategic levy investment | MUSHROOM FUND

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[1] Yun Y-B, Um Y, Kim Y-K. 2022. Optimisation of the bacteriophage cocktail for the prevention of brown blotch disease caused by Pseudomonas tolaasii. Plant Path. J. 38:472-481.

[2] Kertesz MA, Bell TL, Safianowicz K. 2015. Improving consistency of mushroom compost through control of biotic and abiotic parameters. Hort Innovation project MU10021, Final Report.