

# Effect of active prophage on the virulence of *Pseudomonas aeruginosa*



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## Background

Bacteriophage, also called phage, can either help or hinder the survival of bacterial cells. They can infect, reproduce and burst the infected bacterium through the lytic cycle, or integrate into the bacterial genome and become a prophage that is maintained in the bacterial host (lysogen) via the lysogenic cycle (Taylor *et al.*, 2019).

Active phage are abundant in the lungs of patients with cystic fibrosis (James *et al.* 2015) and infecting *Pseudomonas aeruginosa* strains commonly have multiple prophages, but little is known about their relationship. More research is needed to determine how the prophages confer an advantage and drive selection (Davies *et al.*, 2016). This project investigates three bacteriophages from the Liverpool Epidemic Strain (LES) of *Pseudomonas aeruginosa* as they are known to provide an advantage in the cystic fibrosis lung (Winstanley *et al.*, 2009; James *et al.*, 2012).

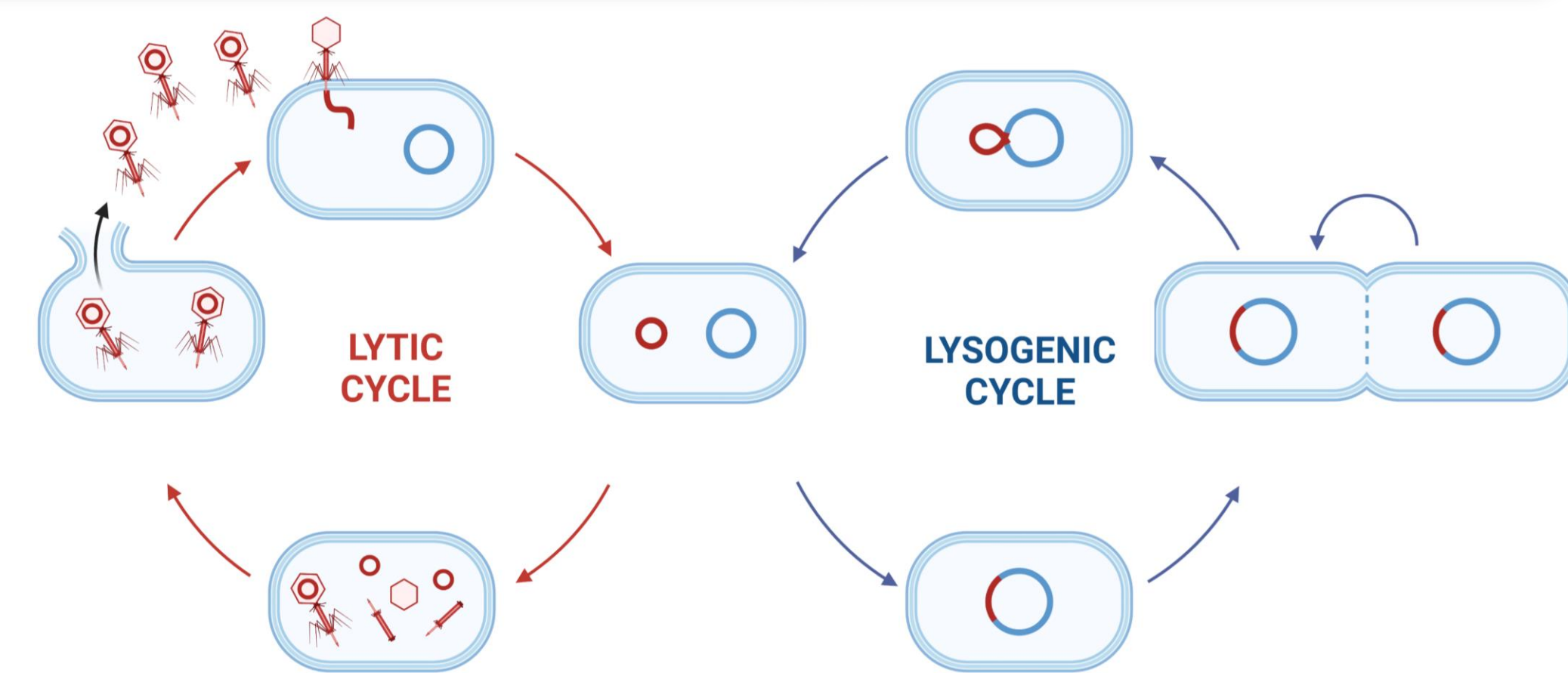


Figure 1: The two possible bacteriophage life cycles when infecting a bacterium

## Results

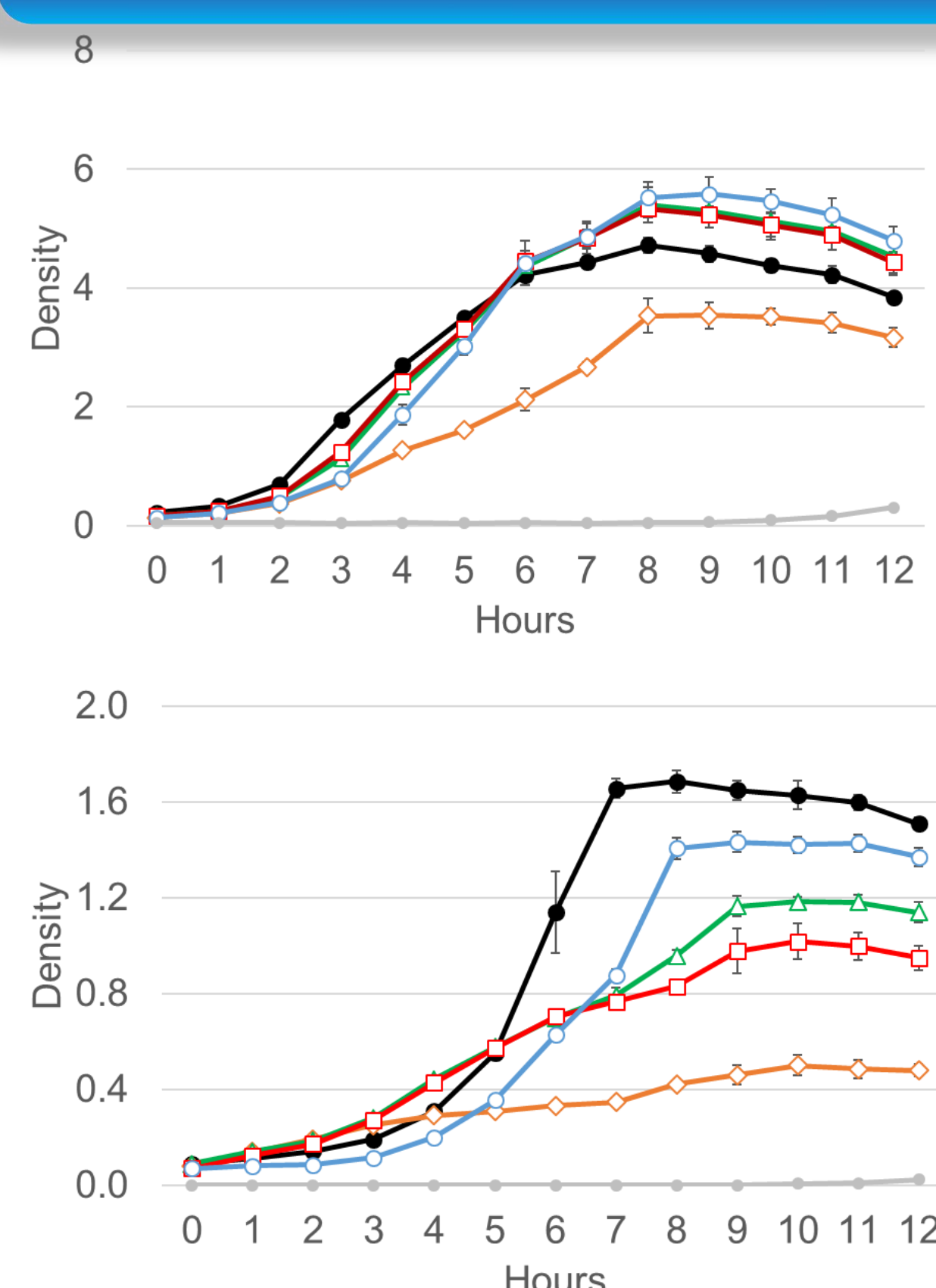


Figure 2: Growth of naive PAO1 vs Lysogens in A) LB medium and B) Minimal M9 medium (0.4% succinate). • WT (Naive), ◊ Phage 2 Lysogen, ▲ Phage 3 Lysogen, ◻ Phage 4 Lysogen, ◌ Phage 2,3,4 triple Lysogen, ● Negative control.

### Growth in rich LB media

- Bacteria with all three phage had the highest growth rate
- Bacteria with prophages 3 and 4 were fitter than naive WT.
- Phage 2 exhibited the slowest growth rate.

### Growth in minimal M9 media

- Bacteria without the phage had the fastest growth rate and reached higher densities.
- Bacteria containing all three phage were fitter than those with one
- Phage 2 exhibiting the slowest growth rate throughout.

### Bacterial load recovered from *G. mellonella*

- The lowest bacterial load was recovered from *G. mellonella* infected with bacteria containing no and all three phage
- The highest bacterial load was recovered from *G. mellonella* infected with phage 3 but considerable variation was observed between larvae

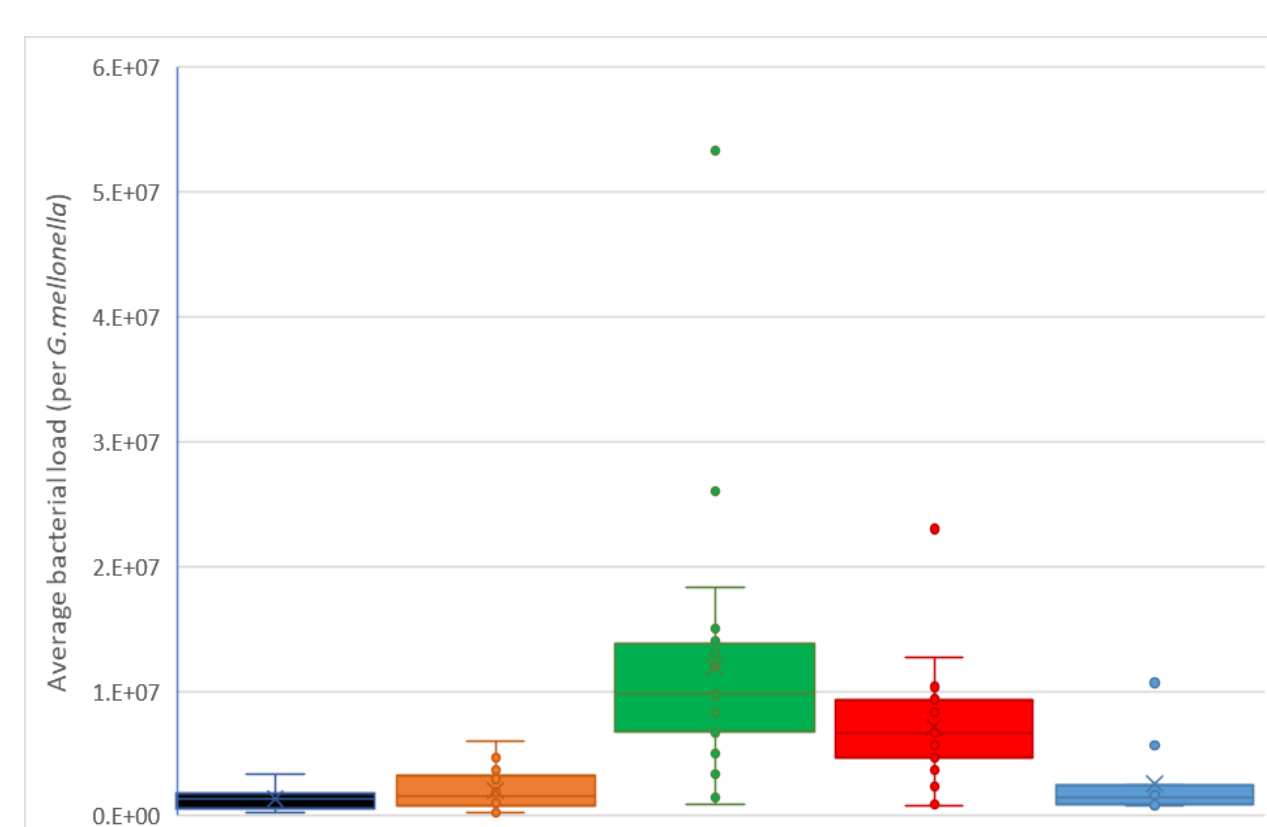


Figure 6: Average bacterial load of WT PAO1 and lysogens found in deceased *G. mellonella*. Each data point shows the mean of three repeats. • WT (naive), ◊ Phage 2 lysogen, ▲ Phage 3 lysogen, ◻ Phage 4 lysogen, ◌ Phage 2, 3, 4 triple lysogen

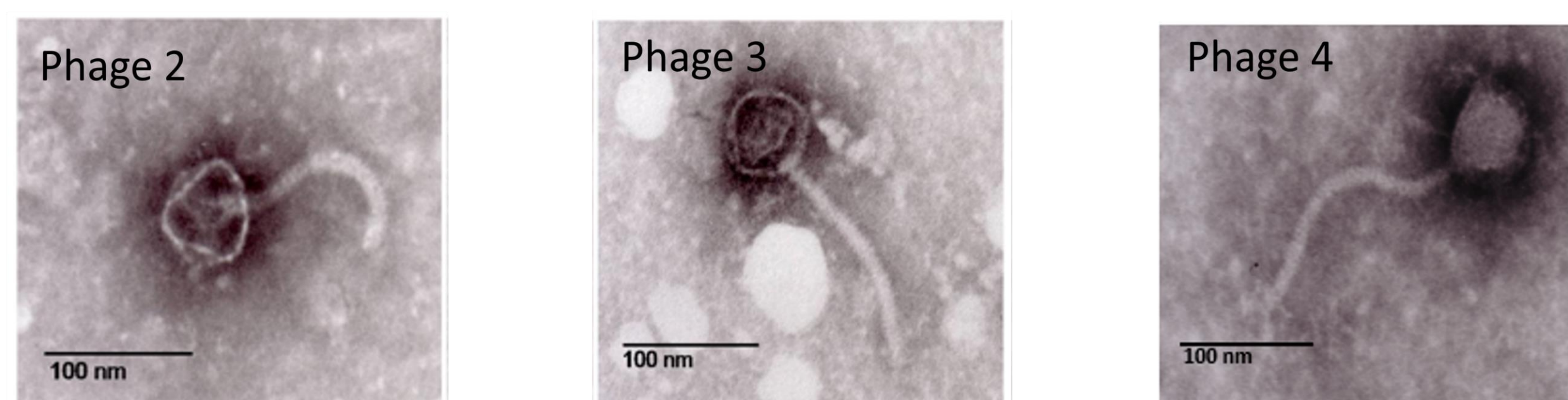


Figure 5: LES Bacteriophage morphologies, all three exhibiting *Siphoviridae* morphology.

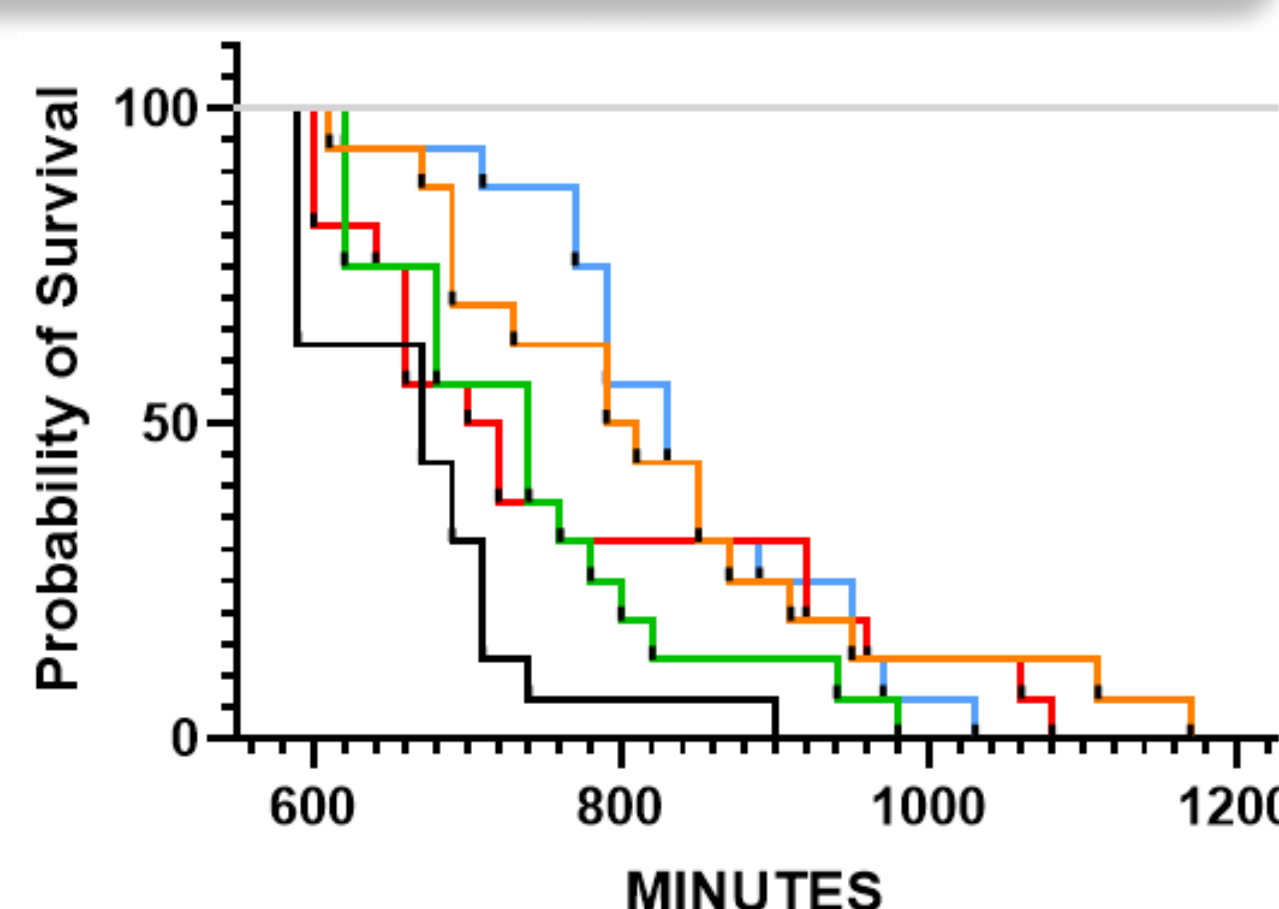


Figure 3: 20 h kill curves of *G. mellonella* inoculated with naive PAO1 and lysogens. — WT (Naive), — Phage 2 Lysogen, — Phage 3 Lysogen, — Phage 4 Lysogen, — Phage 2, 3, 4 triple Lysogen, — Negative control.

### *Galleria mellonella* kill curve

- *G. mellonella* infected with bacteria containing no phage died the fastest
- *G. mellonella* infected with phage 2 lysogen survived the longest
- All strains killed their hosts within 20 hours of inoculation

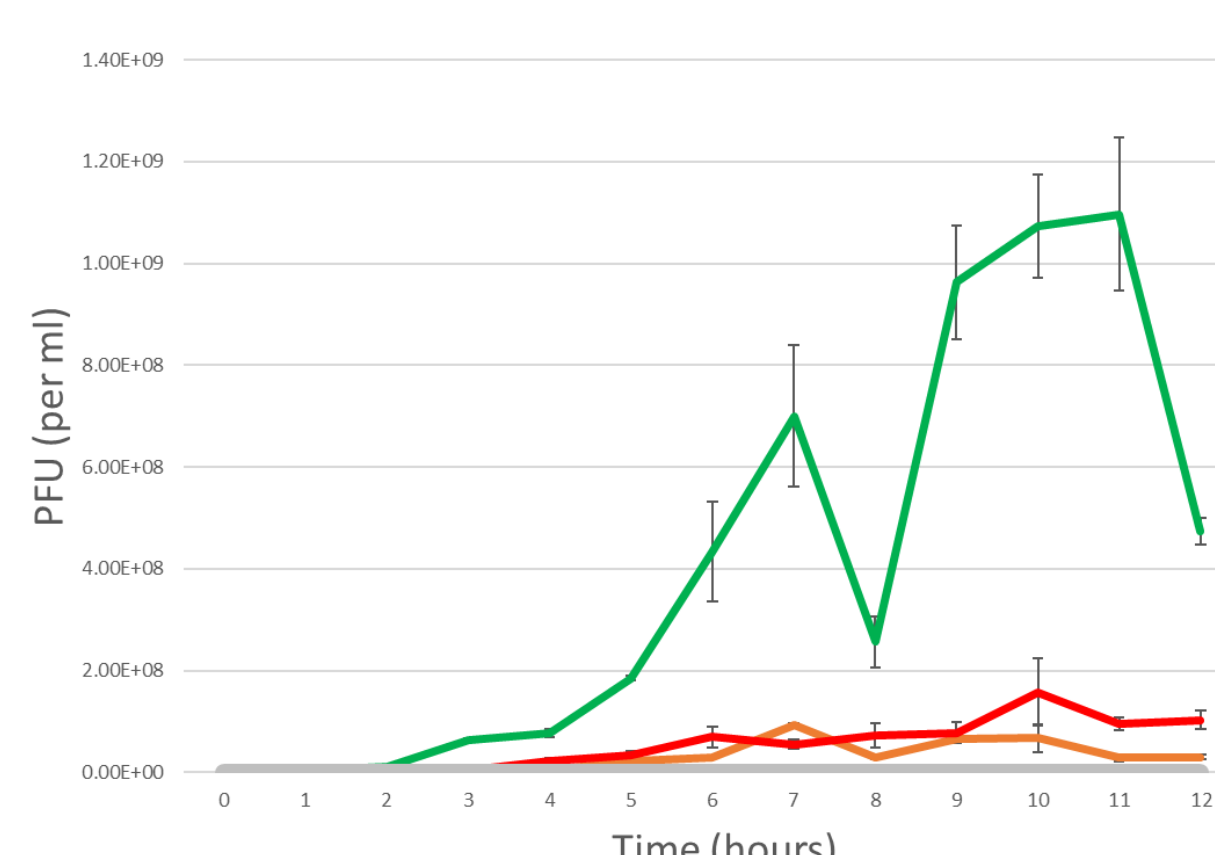


Figure 7: 12h Phage production of Lysogens in A) LB media and B) Minimal M9 media (0.4% succinate). — Phage 2 Lysogen, — Phage 3 Lysogen, — Phage 4 Lysogen, — WT (naive).

## Methods

- Bacteria with and without the phage were grown in rich growth media (LB) and minimal media (M9) at 37°C for 12 hours
- Phage production was measured by performing plaque assays
- *Galleria mellonella* (waxworm caterpillars) injected with 100 µl of 0.5OD culture were monitored for 20 hours at 37°C and frozen once dead
- Frozen *G. mellonella* were individually homogenised in 1ml PBS, diluted, plated on LB agar and incubated at 37°C overnight



Figure 4: LES Bacteriophage plaque morphologies. Filtrates taken from lysogens 2,3 and 4 grown on a lawn of naive PAO1.

### Phage production in rich LB media

- Phage 3 had the highest production rate
- Phage 2 and 4 had similar phage production rates
- Phage 2 and 3 curves follow the same trend
- Phage 2 and 3 production was higher in LB media

### Phage production in minimal M9 media

- Phage 2 reached the highest phage concentration.
- Phage 2 and 3 followed the same basic production trend
- Production of phage 4 was lower than phage 2 and 3
- Phage 4 production was higher in M9 media

## Conclusion

The data indicates a positive interaction between phage and host, with optimal growth in rich media when all three bacteriophage are carried.

Growth in minimal media is limited by prophage carriage, perhaps indicating that the prophages are farming for susceptible hosts.

Bacteria that only contain one phage seem to be less virulent and an increased bacterial load is needed to kill the host.

The data thus far has given an insight into how *P. aeruginosa* interacts with its phage in a controlled environment but more work is needed to see if this interaction would be similar in the cystic fibrosis lung.

## References

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