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How do temperate bacteriophages affect the fitness of *Pseudomonas aeruginosa*?

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Abstract

The Liverpool Epidemic Strain (LES) of *Pseudomonas aeruginosa* is a key opportunistic pathogen and a major cause of morbidity and mortality in cystic fibrosis (CF) patients. It carries distinctive prophages within its genome, which provide fitness advantages that have not been well characterised.
This project explores the dynamics between *P. aeruginosa* PAO1 and 3 LES phages using different models of infection and treatment regimens.

Background

Bacteriophages can either aid or hinder the survival of bacterial cells. They can either 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). It is know that active phages are abundant in the CF lung (James et al 2015) and infecting *Pseudomonas aeruginosa* strains commonly harbor multiple prophages, but little is known about the full extent of 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 active phages of the Liverpool Epidemic Strain (LES) as these have been proven to confer a competitive advantage in the CF lung (Winstanley *et al.,* 2009; James *et al.,* 2012).

Methods

- Naïve PAO1 and lysogens grown in LB and M9 minimal media at 37°C, cultures diluted to an OD of 0.005
- Bacterial growth monitored over 12 hours using growth profiler
- Plaque assays performed to monitor phage production

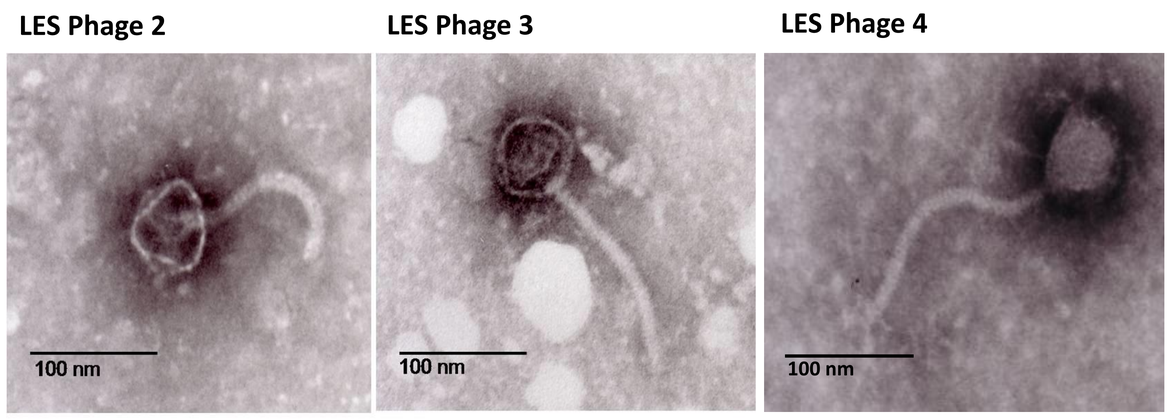

Figure 1: LES Phage 2, 3 and 4, all three exhibit *Siphoviridae* morphology

Results

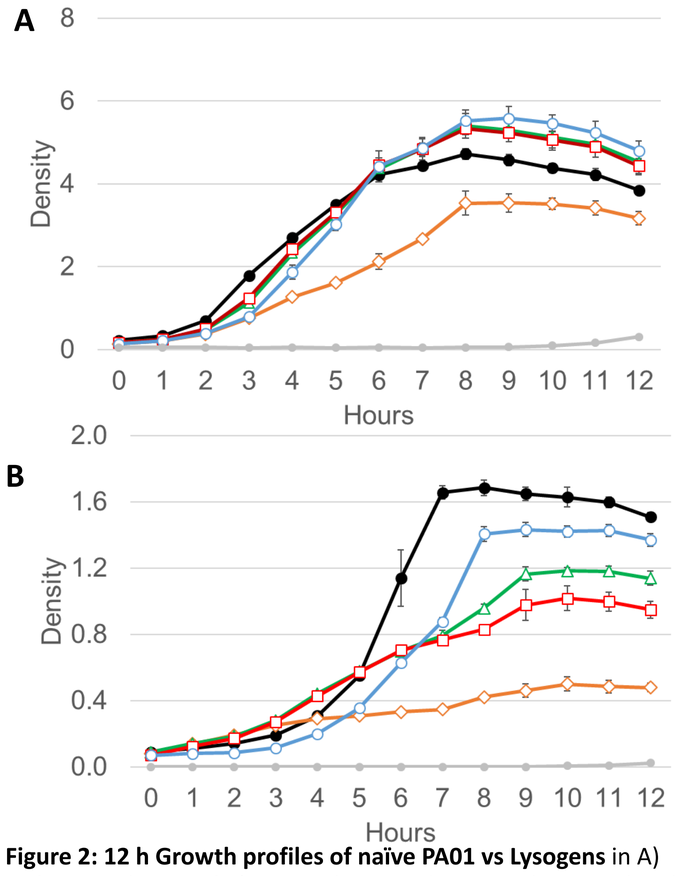

Figure 2: 12 h Growth profiles of naïve PA01 vs Lysogens in A) Rich LB medium and B) Minimal M9 medium supplemented with 0.4% succinate. ● WT (Naïve), ◊ Phage 2 Lysogen, △ Phage 3 Lysogen, □ Phage 4 Lysogen, ○ Phage 2,3,4 triple Lysogen, ● Negative control. Data points show mean of 6 replicates and standard deviation.

A. Growth in LB media

- Triple lysogen had the highest growth rate
- Lysogens harbouring prophages 3 and 4 were fitter than naïve WT.
- Phage 2 exhibited the slowest growth rate.

B. Growth in minimal media

- naïve WT PAO1 exhibited the fastest growth rate and reached higher densities than all lysogens.
- Triple lysogen was fitter than single lysogens.
- Phage 2 lysogen was consistently the least fit, exhibiting the slowest growth rate throughout.

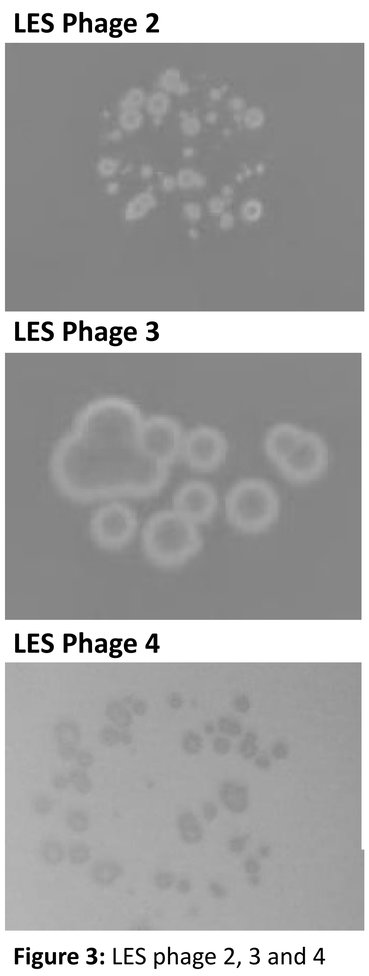

Figure 3: LES phage 2, 3 and 4 plaque morphologies

Phage production

- Phage 4 Lysogen produced the most active phage particles in LB media
- Phage 2 Lysogen production was significantly lower than phage 4, but may reflect reduced host growth rate.

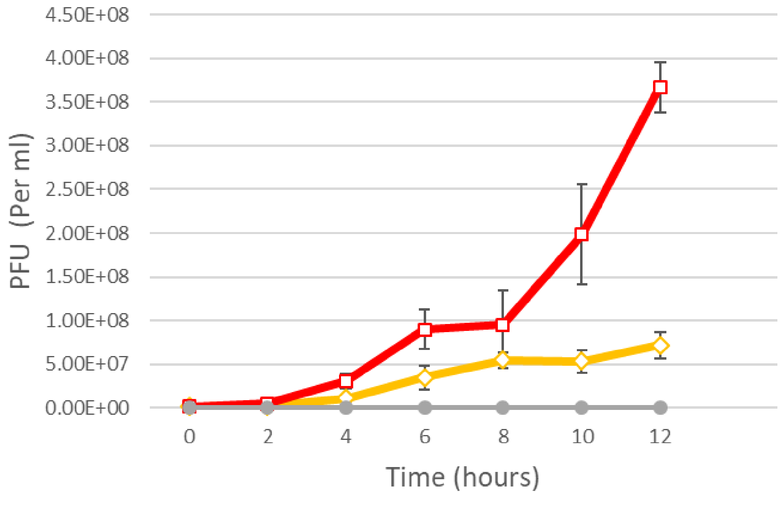

Figure 4: **Phage production of lysogen 2 and 4**. Lysogens grown in LB medium for 12 hours. ◊ Phage 2 Lysogen, □ Phage 4 Lysogen, ● Negative control. Data points show mean of 3 replicates and standard deviation.

Conclusion

The data indicates a positive interaction between phages and host, with optimal growth in rich media when all three phages are carried.
LES prophages affect the growth of their *P. aeruginosa* host differently depending on the nutrient availability and phage type.
Growth in minimal media is limited by prophage carriage, perhaps indicating that the prophages are farming for susceptible hosts.
The data thus far has given an insight into how *P. aeruginosa* interacts with its phage in a controlled environment.

Future Work

Compare growth of lysogens and phage production in pig lung, biofilm and *Galleria mellonella* models imitating the conditions found in a CF lung more accurately. Record changes in phage production and lysogens reaction to antibiotic stress by introducing varying concentrations of Ciprofloxacin into its environment.

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