Understanding and exploiting phage-host interactions for enhanced phage therapy

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1. Introduction

- Uropathogenic *Escherichia coli* (UPEC) are a predominant cause of urinary tract infections (UTIs) globally, affecting 150 million people at a cost of USD$3.5 billion annually[1], which presents a significant health, social, and economical burden.
- UPEC are classified as critical-priority bacteria due to their resistance to third-generation cephalosporin[2], making them an ideal target for phage therapy.
- Understanding the phage-host interactions will enable efficient selection of natural phages and their genetic engineering with enhanced capabilities.
- **The objectives of this work** are 1) to identify UPEC host factors involved in phage susceptibility, 2) engineer phage genomes with heterologous genetic functions to expand their host range against UPEC clinical strains.

2. Antiphage defence systems in UPEC genomes

- We built a bioinformatics pipeline integrating PADLOC-DB[3], DefenseFinder[4], and CRISPRDetect[5] to identify host antiphage defence systems involved in phage susceptibility.
- We identified 77 families of antiphage defence systems in over 400 UPEC genomes from NCBI database (Fig. 1A).
- We discovered some host defence systems are more prevalent in UPEC compared to commensal *E. coli* (Fig. 1B), and that they seem to encode more antiphage defence systems per genome (Fig. 1C).

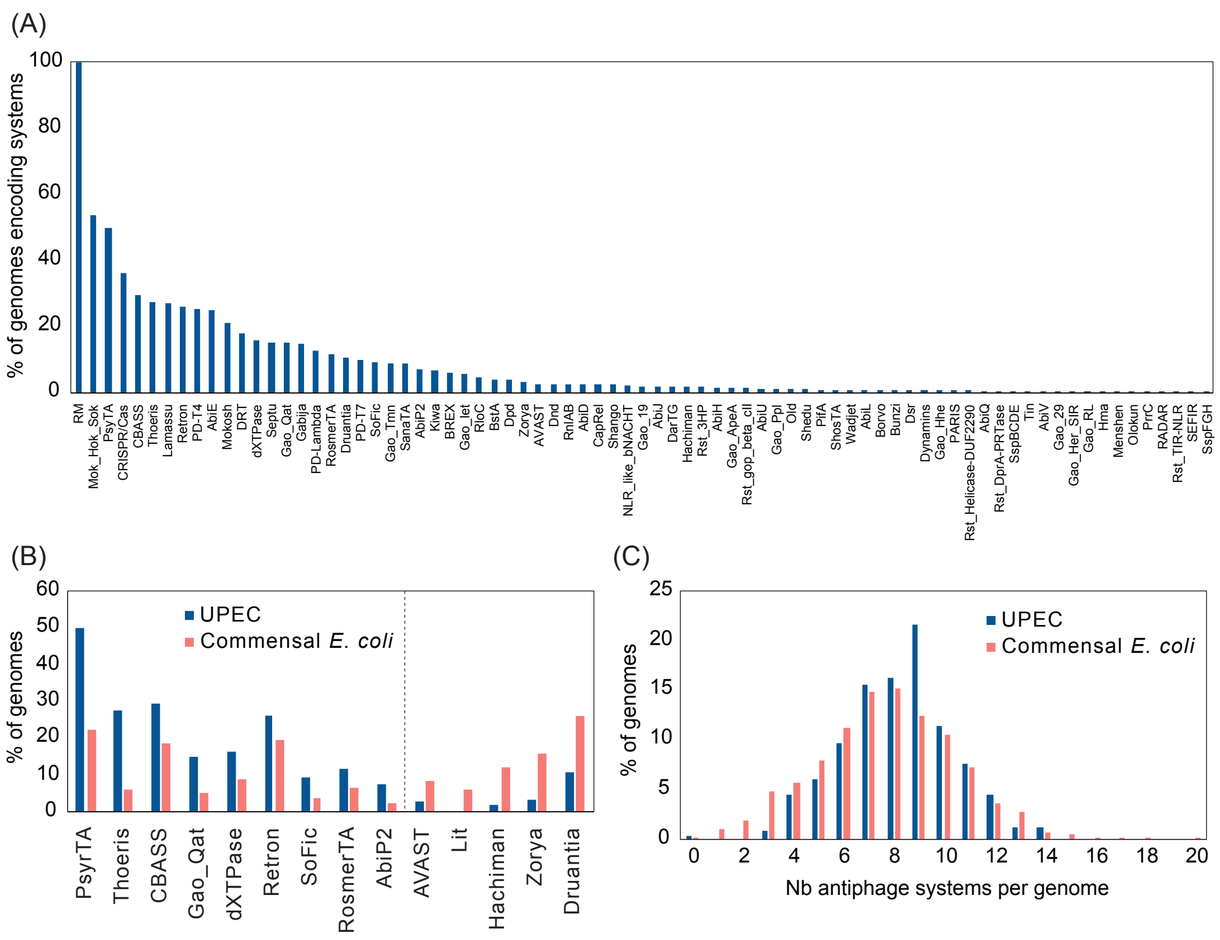

FIG 1 Families of antiphage systems in UPEC genomes. (A) Frequency of systems in UPEC genomes (n=413). (B) Prevalent systems in UPEC and commensal *E. coli* genomes (n=2920). (C) Distribution of total number of antiphage systems per genome.

3. Engineering phage to expand host range

- Newly isolated phage vB_EcoM_SHAK9454, a member of *Autographiviridae*, is an ideal candidate for phage therapy to treat UTIs due to its short life cycle and large burst size. Also, its T7-like genome enables easy manipulation.
- We measured its attachment efficiency againt 36 UPEC clinical strains, and we found that most of the failed infection events could be attributed to failed adsorption (Fig. 2), with the action of host defence systems likely accounting for the remaining cases.

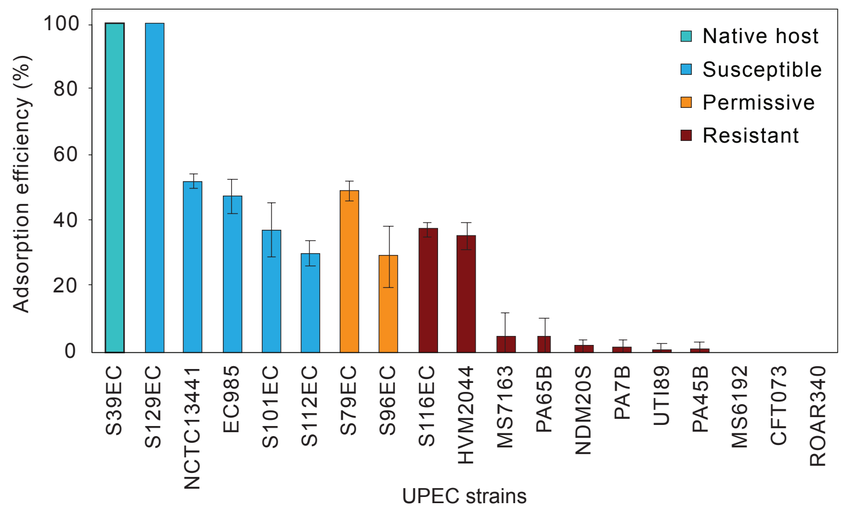

FIG 2 Efficiency of vB_EcoM_SHAK9454 in attachment to UPEC clinical strains. Exponentially growing host was infected with vB_EcoM_SHAK9454 at MOI < 0.01, and the phage/bacteria mixture was incubated at 37 °C in LB for 10 min (n=3).

- We propose to assemble chimeric tail fibres and tail spikes to alter or expand this phage's host range (Fig. 3).
- In parallel, we are developing methods to clone phage into yeast to enable their manipulation and augmentation with heterologous genetic functions.

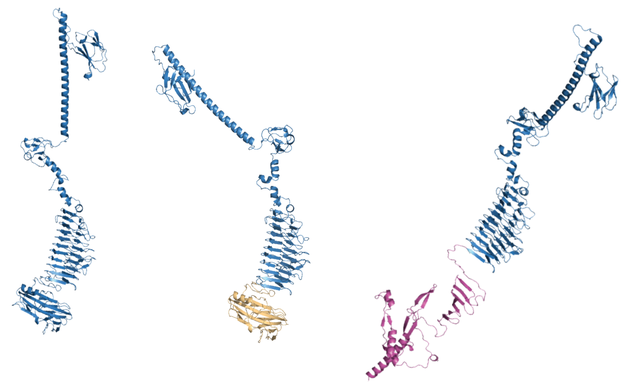

FIG 3 Structures of chimeric tail fibre and tail spike proteins. vB_EcoM_SHAK9454 tail fibre protein (left) serves as a template, and its receptor binding domain is replaced by those of other phages (centre and right). Structural modelling by ColabFold[6].

4. Understanding host requirements for phage replication

- Small heat shock proteins (sHsps) sequester partially folded proteins for future recovery and refolding, assisted by ATP-dependent folding chaperones[7,8] .
- Two sHsps, IbpA and IbpB, are highly upregulated during φX174 infection[9].
- **The objective of this work** is to determine if IbpA/IbpB are necessary for φX174 replication.

Methods

- We used a hybrid approach of CRISPR interference (CRISPRi)[10,11] and genomic knockouts to disrupt *ibpA/B* genes.
- We performed *in vitro* bacterial killing assay to assess φX174 virulence, and one-step growth experiment to measure its burst size and latent period.

Results

- We did not find significant difference in φX174 virulence, burst size, or latent period between wild type *E. coli* C122 and C122Δ*ibpA/B* single knockout strains (Fig. 4A). Similarly, φX174 replication was unaffected by knockout/knockdown of *ibpA* or *ibpB* or both (Fig. 4B).
- sHsps are known to stabilise cell membrane through interactions with membrane lipids[12-14]. We propose that IbpA/B may provide transient protection to *E. coli* cell membrane integrity (Fig. 5), but they are ultimately overwhelmed by lysis protein production and burst of phage progeny.

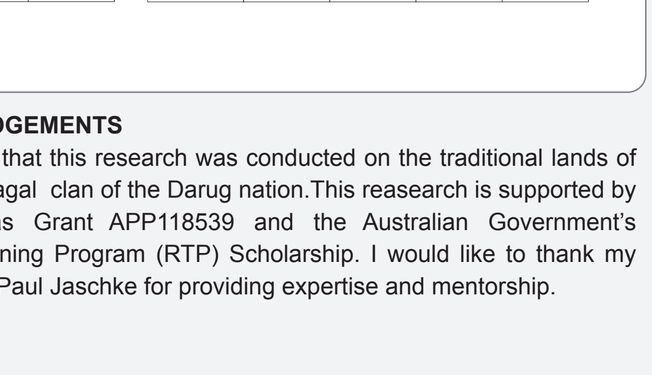

FIG 5 Proposed role of IbpA/B during φX174 infection.

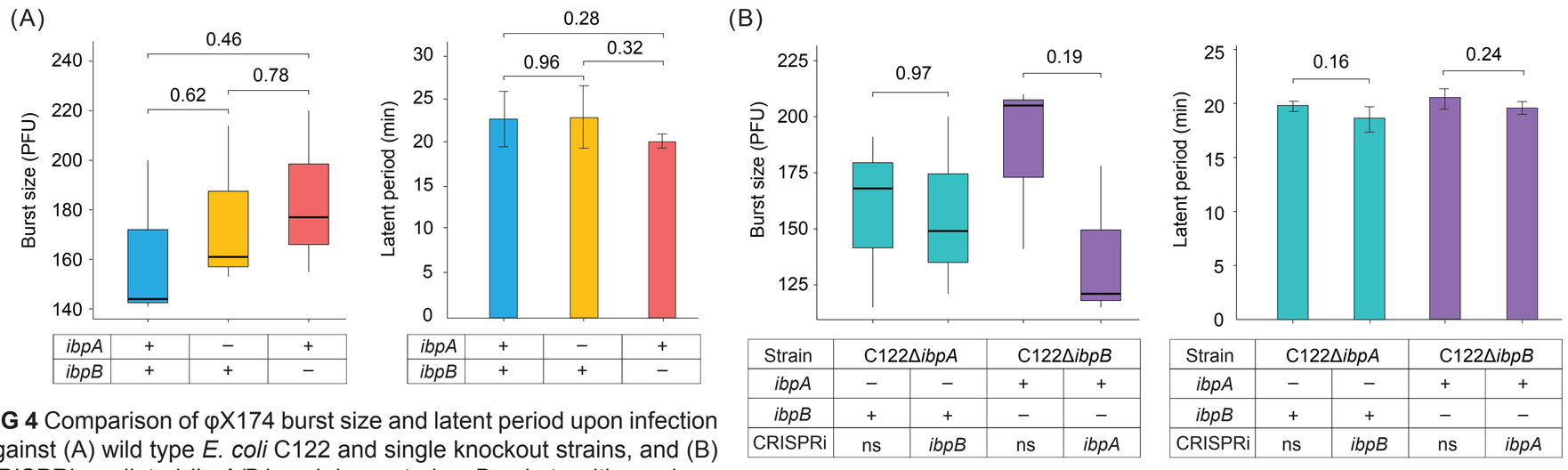

FIG 4 Comparison of φX174 burst size and latent period upon infection against (A) wild type *E. coli* C122 and single knockout strains, and (B) CRISPRi-mediated *ibpA/B* knockdown strains. Brackets with numbers above refer to Student's two-tailed t-test p-values.

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