Characterization of Phage Resistance Mutations in Vancomycin Resistance *Enterococcus*

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Abstract

Enterococcus spp. are commensal bacteria and opportunistic pathogens that may colonize the gut or infect the host and are commonly acquired in hospital settings. Infections with multidrug resistant strains of this genus, most commonly Vancomycin resistant *Enterococcus* (VRE), prove difficult to treat using traditional antibiotic therapies. These strains represent a growing threat and resulted in 54,500 hospitalizations in 2017. As a result, alternative therapies are being explored to treat these infections, including the use of bacteriophage therapy. Bacteriophage therapy involves treating infections with lytic phages that infect and kill target bacteria. Bacteriophage therapy offers several theoretical advantages over traditional antibiotic treatments including better specificity, self-dosing behavior, and variety of phages with diverse host targeting systems. However, like with antibiotics the development of resistance to a given phage treatment remains a concern. Phage resistance can occur through mutations that alter phage binding domains or otherwise disrupt phage propagation. We have isolated 16 mutants of a VRE strain (VRE27) that acquired phage resistance through unknown mechanisms. These mutants were isolated either from *in vivo* experiments where VRE27 colonized mice were treated with phage or during *in vitro* phage culturing. The goal of this project is to identify and characterize the specific mutations imparting phage resistance to the VRE27 mutants.

Bacteriophages

Bacteriophages are viruses that infect bacterial hosts. They fundamentally consist of a protein capsid that houses nucleic acid, a tail sheath, and tail fibers that bind to bacterial host receptors. Because phages rely on bacterial machinery to replicate, the number of phages is proportional to the amount of host bacteria in that environment. Therefore, unlike with fixed doses of antibiotics the number of phages will decrease as the infection is cleared.

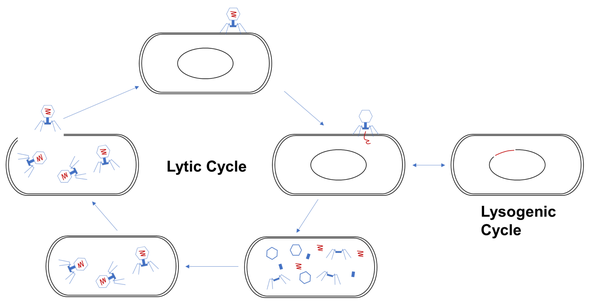


Figure 1. Diagram illustrating the phage lifecycle. Phages first bind to host receptors using their tail fibers. They then inject their genetic material into the host where it is either integrated into the host genome and replicated (lysogenic cycle) or hijacks cellular mechanisms to produce more phage particles (lytic cycle). In the lytic cycle the replicated phages are assembled in the host and eventually lyse open the cell. This kills the bacteria and introduces more phages into the environment to repeat the cycle.

Phage Resistant VRE Mutants

Figure 2. Scanning electron microscopy images of myoviridae (A) and siphoviridae (B) phages taken from PhagesDB[1]. These two families differ predominantly in their tail region, where myoviridae has a short, contractile tail and siphoviridae has a long, flexible, non-contractile tail. Of the phages used for this project ϕ45, ϕ46, and ϕ47 belong to the siphoviridae family while ϕ19 and ϕ53 are members of the myoviridae family.

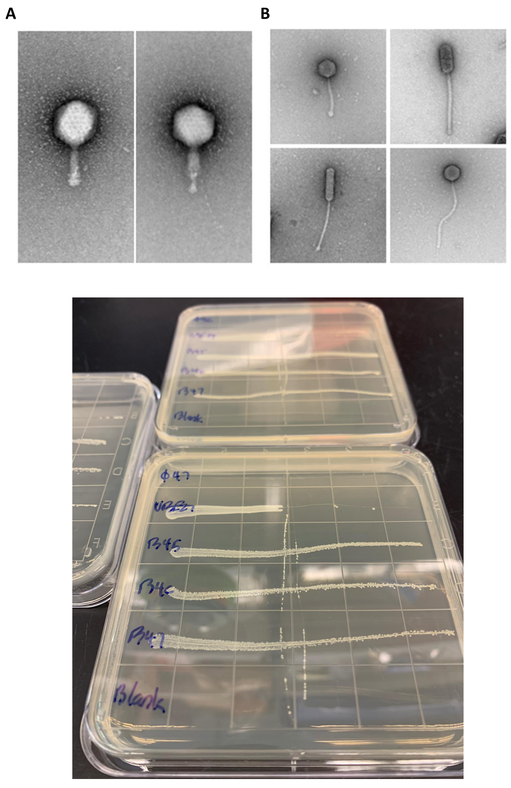
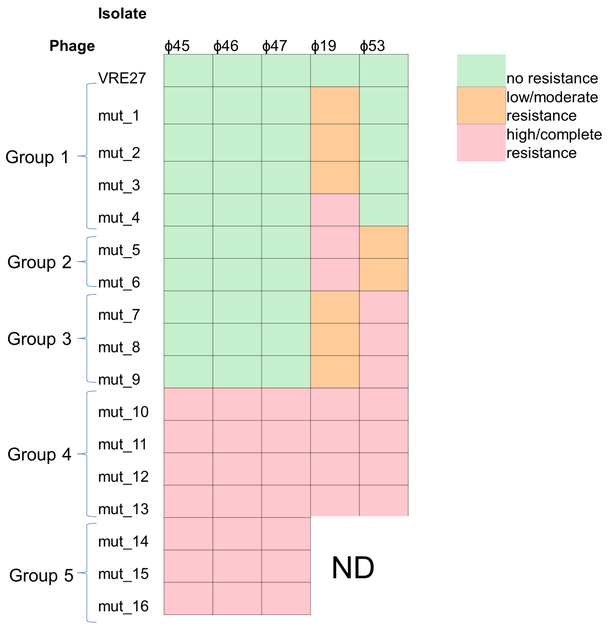


Figure 3. Example cross streak assay used to test for phage resistance. Phage was streaked down the middle of the plate and drops of VRE liquid culture were placed on one side of the phage line. Using a sterile instrument, the liquid culture was dragged across the line and through the phage in one motion. Resistant strains show growth past the phage line, while non-resistant strains won't grow beyond that point. The mutants pictured here were isolated through in vitro phage culturing. All three (group 5 in figure 4) were found to be resistant to phages ϕ45, ϕ46, and ϕ47. While these three weren't tested against phages ϕ19 and ϕ53, it is likely these mutants will be preferentially resistant against siphoviridae phages based on the patterns observed in other phages.

Resistance Mutation Candidates

Figure 3. Resistance profile of all isolated phage resistant VRE mutants. Mutants were categorized into groups based on patterns in their resistance profiles obtained from cross streak assays.



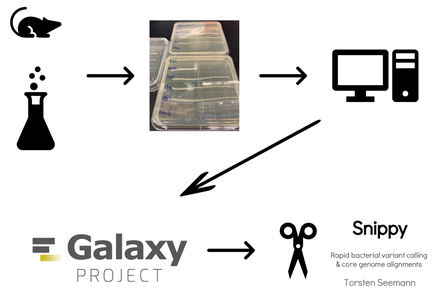
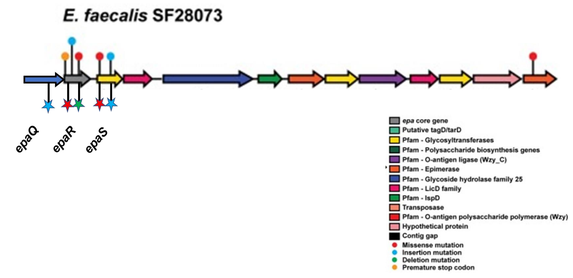


Figure 5. Diagram depicting the overall pathway for mutant discovery and analysis. After collecting and identifying the phage resistant mutants, the genomic DNA was purified and sent for sequencing. The Galaxy bioinformatics platform was used for analysis. Snippy, a Galaxy supported tool, was used for both genome alignment and variant calling.

Figure 6. Gene map of the *Enterococcus faecalis* SF28073 (VRE27) epa region adapted from Chatterjee et al[2]. In this publication, the epa gene cluster was shown to play a role in phage resistance for *Enterococcus.* Of the mutants isolated by our group, sequencing revealed shared epa region mutations in groups 3 and 5. The original published mutations appear as dots. Our mutations are annotated with stars. For groups 3 and 5, it is highly likely that epa mutations are imparting resistance.



Mutants	Mutation	Nucleotide Position	Gene Product
10, 13	C->A missense	2250622	Regulatory protein Spx
11, 13	G->T missense	1786928	Cytidylyltransferase
12	A->G missense	698488	ABC transporter

Table 1. Group 4 top resistance mutation candidates. Group 4 differs from groups 3 and 5 in that none of the resistant mutants contain epa mutations. Additionally, this groups demonstrates complete resistance across all tested phages. For those reasons, these group 4 mutations are of high interest and will be the primary focus moving forward.

Next Steps: Gene Knockout

In the next phase of this project, resistance candidates will be tested using a variation of gene knockout and return of function experiments. This will be accomplished through two phases of allelic exchange with the pLT06 plasmid.

1. Construct an allelic exchange pLT06 plasmid with the gene of interest deleted
2. Clone the gene knockout into the VRE27 parent strain
3. Assess changes in phenotype
4. Construct an allelic exchange pLT06 plasmid with the mutated gene of interest
5. Clone mutated gene into the VRE27 gene knockout strain
6. Assess changes in phenotype

If this method is successful, we hope to use it as a template for identifying resistance mutations in VRE as they arise.

References

1) D. Russel, G. Hatfull. (2016) PhagesDB: the actinobacteriophage database. *Bioinformatics*, 33, 784-786.
2) A. Chatterjee et al. (2019) Bacteriophage Resistance Alters Antibiotic-Mediated Intestinal Expansion of Enterococci. *Infection and Immunity*, 87, e00085-19.