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2022 SEA Symposium Abstract

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Characterization of temperate Gordonia bacteriophages: immunity experiments for subclustering CV phages and potential loss of repressor in the DN phage Ecliptus

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The inaugural SEA-PHAGES class at Case Western Reserve University in Cleveland, Ohio isolated 20 phage capable of infecting Gordonia terrae. Two phage genomes were sequenced, including the DN phage Ecliptus and the DS phage Mareelih. Initial genome annotation indicated that Ecliptus is a temperate phage while Mareelih is lytic. Surprisingly, Ecliptus only produced small clear plaques suggesting that it was a lytic phage. Further bioinformatic analysis revealed that Ecliptus appeared to be missing the repressor gene found in closely related DN phages. To test whether Ecliptus is an actual temperate phage, we are attempting to create an Ecliptus lysogen by varying growth conditions. If successful we will test its immunity with other closely related DN phages.   
  
While traditional methods of placing phages into clusters and subclusters involve whole genome sequence comparisons, we wondered whether superimmunity might be a better method to classify potential subclusters. For these experiments we have employed phages from the CV cluster that contains 41 temperate phages, but have not been subject to subclustering. Bioinformatic analysis revealed that all CV phages have one of two integrase genes. Repressor phams are completely correleated with particular integrase genes even though they are often physically separated in the genome by several genes. In our wet lab experiments, we are evaluating if superimmunity dynamics mirror this classification of rigid integrase-repressor pairing. We are also analyzing whether this integrase-repressor division exists outside of the CV cluster by conducting a census of G.terrae temperate phages, drawing phylogenies based on integrase, repressor, and antirepressor phams, and mapping the difference between the given genes.  
  
In our final project, we are exploring potential synergy between antibiotics and bacteriophages for enhanced host cell lysis. We expect that sub-lethal doses of antibiotics combined with bacteriophage infection will be more effective than antibiotics or phage on their own. We determined the lethal doses to G. terrae of three common antibiotics with different mechanisms of function: Gentamicin (protein synthesis inhibitor), Tetracycline (RNA synthesis inhibitor), and Ciprofloxacin (DNA synthesis inhibitor). The phages selected for this experiment represent both temperate and lytic phages, and are from four different clusters to encompass a variety of genomes. We will plate lawns of G. terrae on agar with different sub-lethal concentrations of antibiotics (25%, 50%, and 75%) and infect the lawns with bacteriophage of known titer. Results will be determined qualitatively through plaque assays. The outcome of this experiment could reveal a general positive relationship between phage and antibiotics on the lysis of host bacteria that could have larger implications for phage therapies in medicine to treat bacterial infections, and a way to combat antibiotic resistance in bacteria.