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2021 SEA Symposium Abstract

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Hunting for Phage Darren During a Pandemic

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The goal of this work was to discover a novel phage and isolate DNA to learn more about what is out in the world compared to previously discovered phages. First, a soil sample was collected with a clean spoon and stored in a plastic bag. After collecting the soil sample, the GPS coordinates, physical description of the soil, the air temperature, the depth of the collection, and a photo of the location were noted. We combined the sample extract with Luria growth media and 0.5 mL of a Bacillus thuringiensis subspecies kurstaki culture. Then, the enrichment was incubated at 30oC and shaken at 180 rpm for 48 hr. The enrichment was tested for presence of phage by spot test of the filtered enrichment sample onto a lawn of Bacillus. The final plaque morphology was spread out and only showed up in the most concentrated sample which was 100. The most prominent phage spot had a solid center with a ring of cloudiness around it.  
 The phage was purified by multiple rounds of plaque assay. The final plaque morphology was a mixed morphology with cloudy, clear and bullseye plaques of 1-2 mm diameter. Webbed plates were created to collect high titer lysate. The titer of the lysate was 4.5 X 10^8 PFU/mL. DNA was purified from lysate using the Promega Wizard kit. The eluted DNA in sterile ddH2O was combined and kept as the purified, concentrated DNA solution at a concentration of 161 ng/ul and was submitted to the University of Pittsburgh for sequencing.  
Our poster will include some perspective on phage hunting during a pandemic. An option was given to do lab indoors or outdoors, due to pandemic precautions, everyone had their individual lab table to work on as well as a virtual partner on zoom to whom they narrated the methods as they performed the lab. Small groups of students were rotated through the class each hour to allow any student who was able to participate in some lab experience. We did each experiment only time, and combined, our work yielded four bacteriophages samples, but only Darren produced DNA of high enough quality for sequencing.