Abstract

There are an estimated 10^8 bacteriophages (phage), viruses that infect bacteria, on earth, thus comprising a significant portion of the biosphere. Of these, a mere 10,733 phages have been isolated and 2,061 phages have sequenced, complete genomes, with even fewer, only 1,073, presently publicly available in the NCBI GenBank database. “Phagehunting” is the process of isolation, characterization, and genomic analyses of phages that infect bacterial hosts. There is considerable interest in using phages as diagnostics and therapeutics of multi-drug resistant (MDR) bacteria. One such MDR bacterium is Mycobacterium tuberculosis, the causative agent of TB, whose phages can be isolated on the easy-to-grow non-pathogenic Mycobacterium smegmatis. Since current phagehunting procedures of Mycobacterium phages all use identical plating conditions, we would like to explore alternative phagehunting procedures to determine if the effects of different types of phages isolated. To date, no phages within the Mycobacterium genus have been isolated that infect Mycobacteria. With these new optimized procedures, we would like to pilot a program, contingent on the success of this study, to expose high school students to the field of molecular and synthetic genomics. We isolated. To date, no phages within the Mycobacterium genus have been isolated that infect Mycobacteria. With these new optimized procedures, we would like to pilot a program, contingent on the success of this study, to expose high school students to the field of molecular and synthetic genomics. We

Methods

3. Purification

Three rounds of purification using the streak plating purification method was necessary to obtain a pure lysate for each individual phage. To do so, 10 μL of the phage was dropped on one side of the plate and streaked out to get individual plaques. 4.5 μL of the top agar with 500 μL of the M. smegmatis culture was poured on the plate such that the agar moved from the point of highest to lowest dilution.

4. Amplification

The phage was now amplified to obtain a high titer lysate. A phage lysate is a concentrated liquid sample obtained by infecting a plate of M. smegmatis with the phage, letting the phage lyse the cells, and adding phage buffer directly to the plate surface of 30 plates to collect the phages. High titer lysate phages yield sufficient quantities of DNA for sequencing. However, prior to this, the number of phages in a sample were to be quantified and expressed as PFU (Plaque Forming Unit)/mL. With an estimated titer, the concentration was manipulated to ensure there would be enough phages to form a “web” pattern. The highest-titer lysates come from plates that appear to have the aforementioned “web” pattern, where individual plaques are nearly visible, but not so densely packed that they cover the whole plate. This indicates that several rounds of phage infection and lysis has taken place, and that the phages and bacteria have the most time and space to produce the highest maximum yield. To obtain this ideal, webbed plate, empirical titer values were tested prior to the 30-plate amplification.

5. Extraction

To prepare for DNA extraction, the amplified phage sample was harvested and concentrated. The buffer from the 30-plate amplification centrifuged to remove agar and cell debris and stirred until NaCl and polyethylene glycol (PEG 8000) were dissolved. The solution was then re-suspended in which phage buffer was added to the phage pellets. The final step prior to extraction of nucleic acids is DNase/Rnase treatment of viral lysates, the purpose of which was to clean the phage lysate.

Results

Figure 1. Screened Soil Sample and purifications of Kalon1. A. The plate of Kalon1’s environmental sample. B. The first purification of Kalon1, the plaque was picked from the first purification plate and was streaked plate.

Figure 2. Kalon1 empirical high titer tests. A. After conducting a spot titer test and assuming that there would be 3000 plaques/plate, 4.5 μL of a 10^-1 dilution was calculated to be an optimal high titer lysate value for the Kalon1 plaque picked using a glass Pasteur pipette. B. Empirically tested 2.3 μL of the undissected, micropipette tip plaque. But clearly, it was not the optimal value, thus I proceeded with the plaque picked with the glass Pasteur pipette.

Figure 3. Environmental Sample Collection Sites. A. The different sampling locations were a small park near the middle school, where 5 samples were collected. Collection sites are denoted using a red marker. Out of these five locations, samples from sites 3, 7, and 8 contain confirmed phase. B. Image of collection site 3. This was under a willow tree and was very wet. C. Image of collection site 5E in Clarkberg. The sample was taken below the layer of mulch and was moderately moist. D. Image of collection site 5E in Gaisberg. The location had an abundance of moss in the area surrounding it and was muddy due to recent rain.

Figure 4. Sample 5E on both 7H9 and LB media. A. Environmental sample 5E plated on 7H9 media, it has three plaques which are all confirmed to contain phages. B. The same environmental sample, 5E, was also plated on LB media, it has two plaques. Both plaques are confirmed to contain phages.

Conclusions

We have recently concluded that the reason the 7H9 culture grows overnight is because it is contaminated. For this reason, we will be testing with carbenicillin, an additive to prevent other bacteria from growing in our culture. With this research, we have shown that there are several alternative methods that improve the results obtained. These methods will greatly accelerate and enhance the efficiency by which we can isolate and cultivate phage. In the future, we will continue to work with Kalon1 to characterize, sequence, and annotate it. Additionally, we will work to compile our own booklet of phagehunting protocols, as the publicly available methods are convoluted and hold serious flaws in their organization. From a broader perspective, we were able to establish phagehunting methods that can be used in MDR-TB testing when working with the non-pathogenic M. smegmatis.

Acknowledgements

I would like to thank everyone who took time out of their busy schedules to help me with this great learning experience. I would like to especially thank Nacyra Assad-Garcia and Lauren Oldfield for helping me immensely throughout this entire process, and Dr. Fouts for his invaluable guidance.

References
