# Isolation, Purification and Analysis of Phages from Soil Sample; infecting cell hosts *Bacillus thuringiensis* and *Streptomyces griseus*

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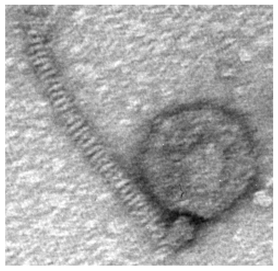


## Abstract

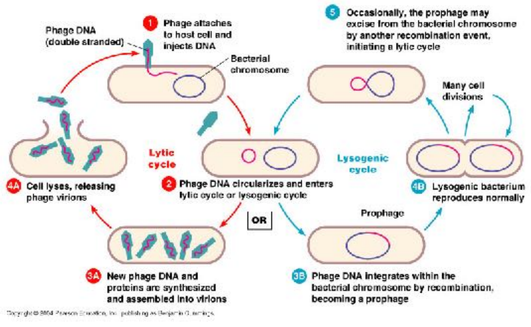
Bacteriophages are thought to represent the major form of life in the biosphere. Although the phage population was originally dismissed by scientists, it was agreed that there is about 1031 bacteriophages worldwide [1]. Using *Streptomyces griseus* and *Bacillus thuringiensis*, we isolated, purified, and electron micrographs were taken of the two phages; DapperPro and Walzer. DapperPro and Walzer originated from University of Maryland College Park and Mount Airy, respectively. DapperPro and Walzer had unique morphology of siphoviridae and myoviridae, respectively.

## Introduction

*Streptomyces griseus:*
- Gram-positive, soil inhibiting and filamentous
- Produces herbicides and pharmacologically active substances
- Produces antituberculosis agent
- Discovered 60 years ago

*Bacillus thuringiensis:*
- Gram-positive, lives in the soil and can survive with and without oxygen
- Important in controlling pests like insects with low effect on humans [1]

- Two major lifecycle parts; lytic and lysogenic
- Bacteria have built resistance to antibodies
- An alternative method to combat bacterial infections is phage therapy
- Phage interacts with bacterium via specialized interactions
- Prophages can be engineered to make bacteria more susceptible to antibiotics
- Samples were taken from local environments
  - Mount Airy
  - College Park
- We will isolate and purify a single phage
- DNA will be extracted and sequenced




## References

Ibrahim, Mohamed A., Natalya Griko, Matthew Junker, and Lee A. Bulla. "Bacillus Thuringiensis." *Bioengineered Bugs* 1.1 (2010): 31-50. Web.
John Travis. All the World's a phage-Viruses that eat bacteria abound and surprise. 2014
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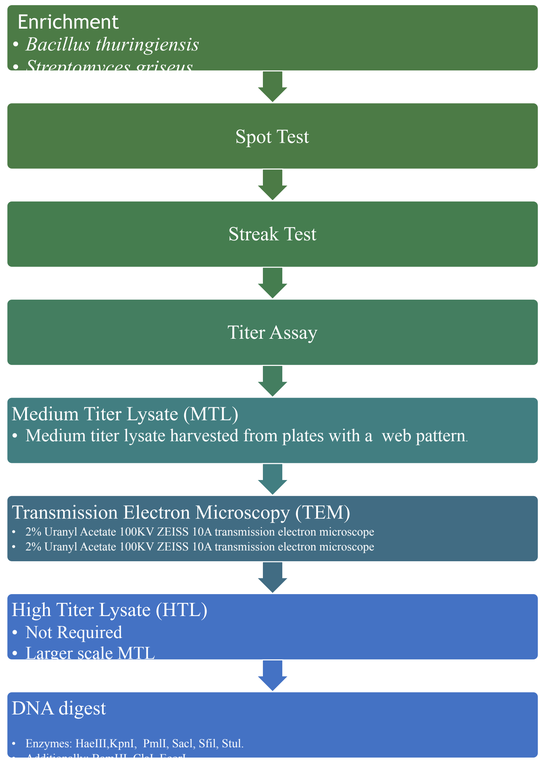
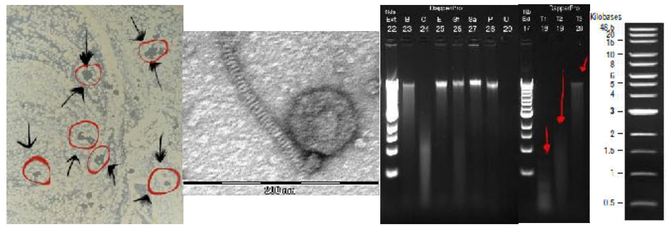
## Methods




## Results

**Figure1:** Panels A, B and C show plaque morphology/turbidity, morphotype of DapperPro and digestion of DNA using different restriction enzymes.

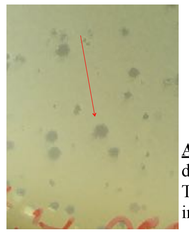



**A** shows plaque average size and turbidity after a 48h incubation at 30 C. Average size is 0.5mm, with a clear appearance.

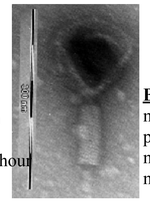
**B** shows the electron micrograph of DapperPro that reveals to be a siphoviridae

**C** uses different Restriction Enzymes:**B=** BamHI, **C=** ClaI, **E=** EcorI, **SfiI**, **Sa=** SacI, **P=** PmlI, **T1=** HaeIII, **T2=** KpnI, **T3=** Stu. Lane 29 represents undigested phage-DNA; lane 22 DNA-ladder.

**Figure2:** Panels A shows plaque morphology/turbidity and panel B shows morphotype of Walzer.



**A** Plaques are between 5-8 mm in diameter with a clear turbidity, The plaques show up with in a 24 hour incubation period.




**B** Electron micrograph of Walzer phage with morphotype myoviridae.

## Results

The results section presents a side by side comparison of the finding of our collaborative effort in isolating phages using our different host cells. All these results are determined using the pictures included in the previous section part if the poster.

| | Bacillus thuringiensis | Streptomyces griseus |
|---|---|---|
| Plaque morphology | Round clear plaques, size around 3-8mm. Plaques show up after a 24 hour incubation period | Round, clear plaques, average size of the plaque was 0.5 mm. Optimum and consistent plaque size was obtained after a 48 h incubation at 30 C. |
| Turbidity | Relatively clear, with slight turbidity | Plaques revealed to be pretty clear all the way around. |
| Morphotype | Myoviridae | Siphoviridae |
| Head width | 80 nm | 68.7 mm |
| Head length | 89 nm | 78.1 mm |
| Tail length | 180 nm | 187.5 mm |
| Tail width | 23 nm | 9.4 mm |
| A260/A280 | DNA was isolated from a 1.97 X $10^9$ pfu/ml titer 100 µl for 2 samples at 1.74 and 1.66 | DNA was isolated from a 2.5 X $10^8$ pfu/ml titer 100 µL (1.83) and 30 µ (1.74). Average: 1.59 for 130µL |
| Concentration | 100 µl for 2 samples at 24.1 ng/µl and 21.1 ng/µl | 100µL concentration: 43.0 ng/µL and for 30µL concentration: 40.0 ng/µL. Concentration for 130µL is 83.0 ng/µL. |

## Discussion

- We were able to successfully isolate two phages from soil samples; DapperPro from the University of Maryland College Park, and Walzer from Mount Airy; using and *Bacillus thuringiensis* respectively.
- DapperPro revealed to be a siphoviridae and Walzer a myoviridae.
- The turbidity of the plaques from DapperPro suggests that the phage follows the lytic lifecycle. Further tests remain to be done for absolute certainty.
- For experimentation purposes, restriction enzymes that are know to work with *Bt* were used on our *Streptomyces griseus*. Phage (DapperPro) and some bands were observed. Compared to other phages that remained uncut by the same *Bt* enzymes, Panel C of figure one confirms the uniqueness of our phage.
- Digestion using enzymes HaeIII, KpnI and Stu (showed in figure 1, Panel C), does not show clear DNA bands in any of the digestion lanes. Lanes T1-3 present with a smear, which in turn makes it harder to approximate the size of the DNA.
- There are a lot of possible reasons why we would have a smear instead of a DNA band. The DNA could have been degraded. This set of experiment was ran a day after the other digestion data.
- The plaques caused by Walzer were clear with slight turbidity and suggests a lytic lifecycle. Further tests remain to be done to support this claim.

## Future Works

The following experiments remain to be done:
- PCR analysis to obtain both phages genomic DNA through to acquire a higher concentration of DNA
- QC gel analysis for both phages to acquire quality of genomic DNA
- Host range tests on both phages
- Super-infection assays on both phages to determine phage lifecycle
- Cluster analysis
- Sequence both phages genomic DNA