The isolation of 205 bacteriophages on *Microbacterium foliorum*: Some struggles and some successes

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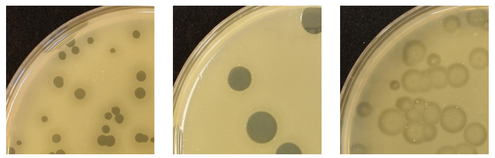
INTRODUCTION

After seven years and the isolation of 756 phages on the host *Mycobacterium smegmatis*, we were interested in discovering new phages on a different host in order to contribute to a better understanding of phage diversity. In our Phage Discovery Lab course, BIOL 105L, we decided to use *Microbacterium foliorum* NRRL B-24224 SEA as a host for potential phages from local environmental samples.

We purified and characterized 205 phage isolates from enrichment cultures of soil, grass, acorn, moss, bark and water samples. The phages were propagated at room temperature (22-25 deg C) during isolation, purification, and amplification. At this temperature, lawns and plaques could be observed after 24 hours, but plaques continued to grow in size over the next 3-4 days.

We had good success finding phages on *M. foliorum*. Positive spot test results were obtained from 56% of all filtered, enriched samples. The phages produced a variety of plaque morphologies, small and large plaques, clear and turbid, crisp or fuzzy edges, and sometimes bullseyes.



Flooding webbed plates from plaque assays of serial dilutions failed to yield lysate of sufficiently high titers. Many students failed to isolate sufficient quantities of DNA for restriction analysis from their lysates, regardless of having sufficiently high titers.

We set out to troubleshoot DNA isolation with the hopes of learning how to isolate better quality DNA from these phages.

RESULTS

A high fraction of phage lysates produced poor DNA

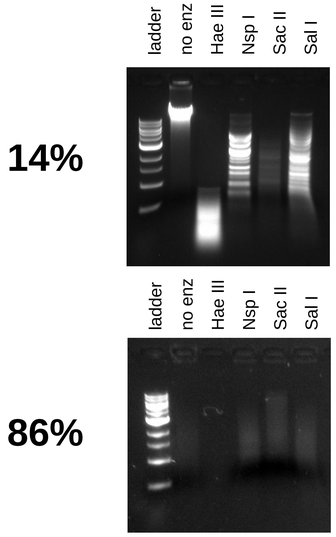


Figure 1. Representative agarose gel results of *Microbacterium foliorum* phage DNAs. 500 ng DNA was digested with restriction enzyme, then loaded into 1% agarose gels with SYBR safe DNA stain. Gels were run at 100V for 60 minutes. Percentages were calculated using a random sampling of 121 phages. The "failure rate" for DNA was much higher this year than in the past with phages isolated on *Mycobacterium smegmatis*. What was going on?

15 uL of lysate on an agarose gel strongly predicts DNA quantity in the DNA extraction step

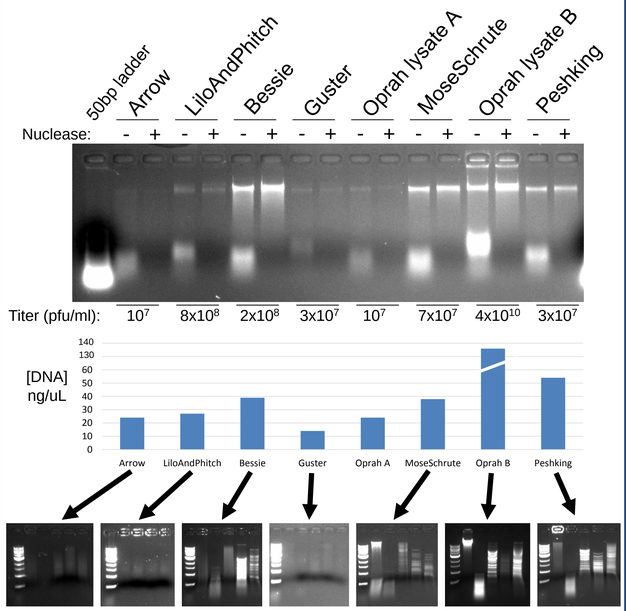


Figure 2. Crude lysates were observed for DNA (and RNA) content. 15 uL lysate was treated with or without the standard nuclease mix at 37 °C, 10 min. EDTA and 6X loading dye was added, then incubated at 65 °C, 5 min, then loaded to an agarose gel with SYBR safe stain. DNA was extracted from lysates with a Promega kit according to the Phage Discovery Guide. DNAs were digested with restriction endonucleases as in Figure 1. Some gel images are overexposed in order to detect DNA in the lanes.

When lysates were treated with nucleases, the DNA of some phages was altered

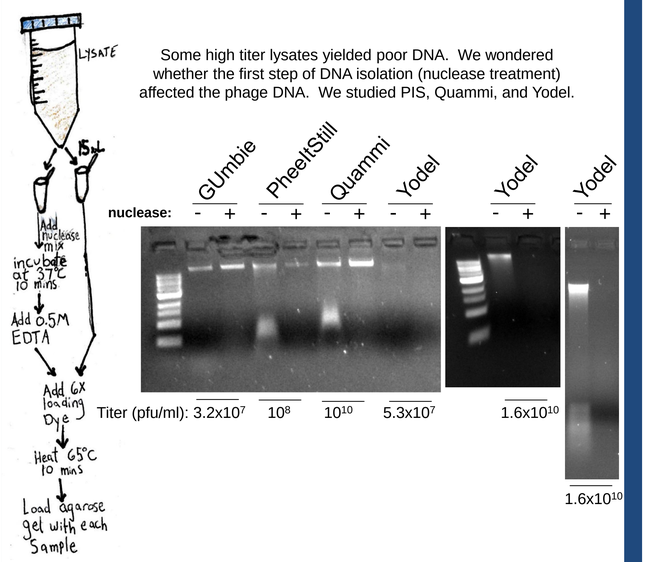


Figure 3. Crude lysates, treated with nucleases. Lysate from *M. foliorum* phages PIS, Quammi, and Yodel and one *M. smegmatis* phage, GUmbie, were prepared using the protocol above. The samples were loaded into a 0.8% agarose gel, run for 45 min, 100 volts. High molecular weight DNA in GUmbie and Quammi was not affected, but in the PIS lysate, this DNA was reduced in quantity, and it was apparently eliminated from the Yodel lysate.

The DNA of some *M. foliorum* phages is not full length when nuclease step is included in purification protocol

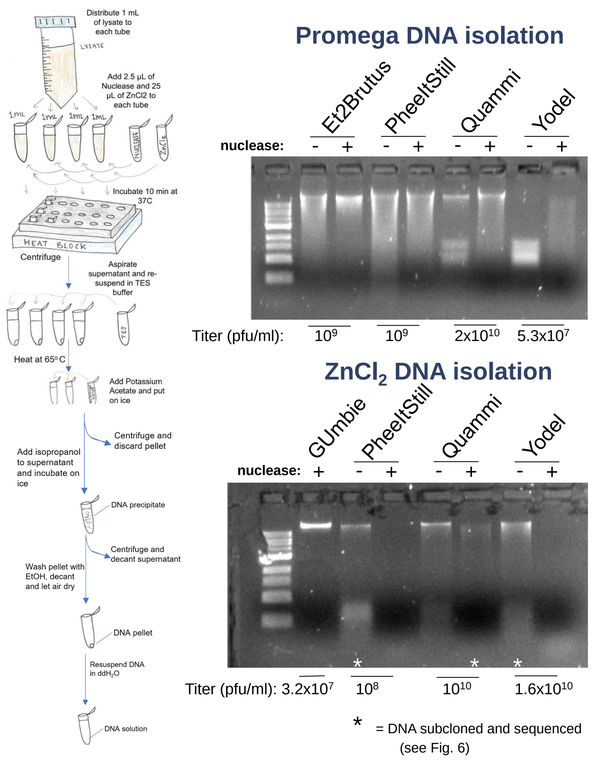


Figure 4. DNA isolation using Promega and $ZnCl_2$ protocols. DNA from three *M. foliorum* phages and from two *M. smegmatis* phages was isolated using the standard Promega protocol and using the $ZnCl_2$ protocol depicted above. Lysates were either treated (+) or not treated (-) with nuclease mix in the first step of the protocols. Based on A_{260}, an apparent 500 ng of DNA was loaded in each well.

Phage morphology does not suggest a reason for lysate sensitivity to nucleases

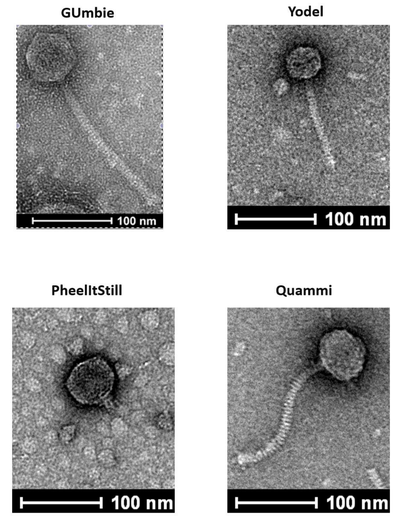
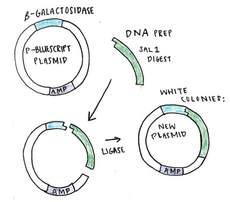


Figure 5. Transmission electron micrographs of phages. Lysates were stained with uranyl acetate and imaged. Yodel is among the smallest phages isolated at Gonzaga. Pheelitstill exhibits a podoviridae morphology.

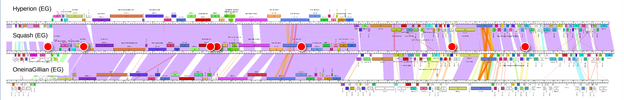
Quammi is likely a cluster EG phage; PIS and Yodel DNA preps ($ZnCl_2$) contained *M. foliorum* DNA

We were curious to find out what kind of phages we had isolated. We cut the DNA preps with Sal I, ligated the fragments to a cloning plasmid, transformed *E. coli*, and did Sanger sequencing on 12 plasmid clones from each phage DNA prep.



Quammi

Seven clones aligned with Hyperion and/or Squash (cluster EG), with 85-100% identity ($E = 10^{-12} – 0$). Red dots ● show locations along the Squash genome.



PheelItStill and Yodel

12 clones each (100%) aligned with *M. foliorum* str. 122 with 80-90% identity ($E < 10^{-50}$). Alignments occurred throughout the *M. foliorum* genome. No phage-specific proteins were found by BLASTx.

A partial, but typical, alignment:

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Query  379     GACTGTCACCGCGCGCCGGCGCTTCGCAGGACGCGCTCGACATGAGCGGCGACCCATTCC  438
               ||| ||||||  |||||| ||||||||||||||| ||||| || ||||||| |||||||||
Sbjct  396609  GAC-GTCACCCTGCGCCGACGCTTCGCAGGACCCGCTCCACGTGAGCGGTCACCCATTCC  396667

Query  439     GCATCGAACGATCGGCTGCGCAGCAACCAGCGGTGGAAGATCGGTCCGACGAACAGCTCT  498
               ||||||||||| |||||||| |||| ||||||||||||||| ||| |||||||||| | ||
Sbjct  396668  GCATCGAACGGTCGGCTGCGAAGCAGCCAGCGGTGGAAGAGCGGCCCGACGAACGGTTCC  396727
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Figure 6. DNA isolated from lysates was subcloned and sequenced. The cloned sequences were aligned with BLASTn or BLASTx to NCBI and phagesDB databases. DNA was cut with Sal I, ligated to pBluescript II SK(+), transformed into *E. coli*, and AmpR colonies were selected. Plasmid DNA isolated from white colonies was Sanger sequenced from a T3 primer. All the clones from the PIS and Yodel DNA preps matched *M. foliorum* str. 122 imperfectly. We suspect that the majority of the clonable DNA from these preps was from the *M. foliorum* host.

CONCLUSIONS

1. We isolated 205 phages on *M. foliorum*.
2. ~86% of the phage lysates failed to yield good DNA.
3. Some failures were likely due to low titer of the lysate.
4. Other failures may be due to the instability of some phage capsids in lysates, leading to susceptibility to nucleases.

- Why does high molecular weight DNA disappear from Yodel lysate when treated with nucleases? Is the large DNA bacterial or viral?
- Why is there apparently so much bacterial DNA in the lysates, relative to phage DNA?
- Is it possible that some of these phages contain ssDNA or RNA genomes?

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