

Mycobacteriophage Interaction

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Introduction I: What are mycobacteriophage?

- Mycobacteriophage, or phage, are like viruses, except that they attack bacteria cells.
- Phage are the most abundant organism on Earth; there are approximately 10^{31} in total.
- Phage consist of a head that encases their double stranded DNA and a tail that is used to pierce bacterial membranes.
- There are two types of phage: lytic and temperate. Lytic phage like a virus, inject their DNA into a bacterial cell where new phage are produced and break out of the cell. Temperate phage however, integrate their DNA into their host bacterial cell's DNA and remain in the cell until an environmental change.

Bacteriophage

Introduction II: What are mycobacteriophage?

- Because phage attack bacteria, their plaques can be found on bacterial lawns. Lytic phage create completely clear plaques, and temperate phage produce plaques with a clear center surrounded by a hazy ring.
- Phage can be used in medicine to help produce antibodies and also in phage therapy. Phage therapy is the idea of using phage as a remedy against bacterial infection.
- Scientists know that phage interact with bacteria through membrane proteins, but it is unknown whether or not phage interact with each other.

Hypothesis: Two phage will interact

- Judging by the fact that phage can almost instantaneously interact with a bacterial cell, I believe that if two stable phage are plated together they will interact. This interaction will be seen in their plaque number and morphology.
- If these two phage are plated together at different ratios to each other and at different dilutions so as to ensure that an optimum environment for interaction is reached, results should be obtained that support my hypothesis.

Methods I: Isolating Phage

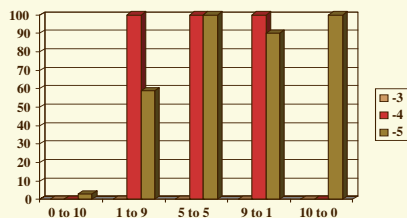
- In order to obtain phage, they must be isolated. The easiest place to find phage is in dark soil. Once a soil sample is obtained it can be mixed with a bacteria, *M. Smegmatis*, and MP Buffer, a buffer solution.
- This solution gets filtered through microscopic filters which separates the phage from the bacteria.
- The now isolated phage is plated with *M. Smegmatis*, and its plaques are picked to ensure that a solution can be produced with a high level of phage. This solution is called 'high titer phage'.
- High titer phage is the optimum result from a phage isolation because it can then be used in DNA sequencing, electron microscopy, and in this case, experimentation.

Methods II: Plating two phage together

- Two high titer phage can be plated together on the same lawn of *M. Smegmatis*. For my first experiment I will be using the phage Chi 12 and D29. The second experiment will use Chi 12 and L5.
- I will plate these phage together a different dilutions. A dilution describes the number of phage present in a solution that will be plated. The Chi 12 and D29 will be plated at -3, -4, and -5 dilutions. The Chi 12 and L5 will be plated at -6, -7, and -8.
- Each plate will have different ratios of phage, such as D29:Chi 12 in ratios of 0:10, 1:9, 5:5, 9:1, and 10:0. The same was done with L5 to Chi 12. The plates will be incubated and results will be recorded 2 or 3 days after the initial plating.

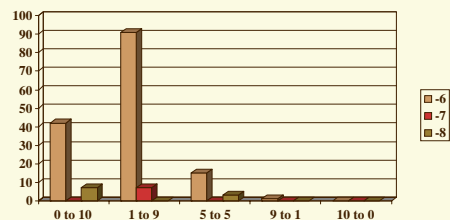
Ratio(D29:Chi12)	0:10	1:9	5:5	9:1	10:0
D29: 0 uL Chi12: 100 uL Smeg: 100 uL MP Buffer: 100uL	D29: 10 Chi12: 90 Smeg: 100 MP Buffer: 100	D29: 50 Chi12: 50 Smeg: 100 MP Buffer: 100	D29: 90 Chi12: 10 Smeg: 100 MP Buffer: 100	D29: 100 Chi12: 0 Smeg: 100 MP Buffer: 100	
Ratio (L5:Chi12)	0:10	1:9	5:5	9:1	10:0
L5: 0 Chi12: 250 Smeg: 100 MP Buffer: 150	L5: 10 Chi12: 240 Smeg: 100 MP Buffer: 150	L5: 50 Chi12: 125 Smeg: 100 MP Buffer: 225	L5: 90 Chi12: 25 Smeg: 100 MP Buffer: 285	L5: 100 Chi12: 0 Smeg: 100 MP Buffer: 300	

Results: D29 and Chi12



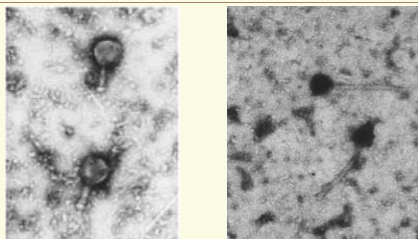
There were no plaques at -3, but both -4 and -5 had interesting plaquing. (100 plaques represents a lacy plate; no bacteria is left)

Results: L5 and Chi 12



Each dilution had a different plaque number.

Evidence: My Phage



These are two of the phage I found. Generally, this is what they look like under an electron microscope.

Conclusion: What happened?

- Overall, my results were very interesting. For instance in the first experiment, D29 created plaques on all of the plates while, Chi12 only plaqued on three of them. In equal proportions both phage obliterated the bacteria, but in uneven ratios the phage didn't always produce a lacy plate.
- In the second experiment, Chi 12 overtook L5 in plaquing, but was greatly affected when higher amounts of L5 were added. L5 didn't seem to plaque at all, even when plated alone.
- Generally, my hypothesis was correct because it can be assumed that phage do interact in some manner. Chi12, D29, and L5 were all constant phages whose expected plaquing manners were disrupted based upon their mixture with another phage. None of the phage plaqued normally when they were plated with another phage at any ratio.

Future Studies: Phage of the Future

- I would like to research phage therapy and try and discover how they could be useful in the field of medicine. Perhaps phage are the antibiotics of the future.
- Because phage evolve so quickly, we can use their genomes to study how different phage are related and compare their DNA.
- I would also study other phage interactions and if possible study their plaques under a microscope to study not only number, but morphology more closely.



Works Cited

- Antibiotics- What next? <<http://www.bbc.co.uk/education/asguru/generalstudies/sciencetechnology/18antibiotics/antibiotics08.shtml>> [Accessed on: 17 May 2005].
- Hatfull, Graham F. Molecular Genetics of Mycobacteriophages. ASM Press; Washington D.C. 2000.
- Pedulla, Marisa L. et al. Origins of Highly Mosaic Mycobacteriophage Genomes. Cell, 2003.
- Berg, Jeremy M. Biochemistry. W. H. Freeman and Co.; New York.