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Graham Hatfull's Lecture Part 1:
Bacteriophages: What are they?

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1. Keywords and Terms

Bacteriophages, plaque, plaque test, lysate, head, capsid, tail, Caudovirales, Myoviridae, Podoviridae, Siphoviridae, lytic growth, adsorption, lysis, lysogeny, temperate, turbid plaque, induction, prophage, immunity, superinfection, site-specific recombination, integration, excision

2. Lecture Notes

Introduction: Discovery and morphology of bacteriophage

Between 1915-1918, D'Herelle and Twort independently discovered phages as killers of bacteria. Serial dilutions of bacterial "killing agent" led to individual circular areas of no

bacterial growth when plated on agar along with the appropriate host. Each of these "plaques" is the result of plating a single virus particle, which then infects, replicates in, and eventually kills the host cells. Thus, the plaque is an area where all, or most of, the cells have been killed and it can contain 108 or more phage particles. The plaque stops getting larger once the cells stop growing. Further explorations by D'Herelle showed that when lysates were filtered, they retained their infectious properties. When the filtrates were examined by light microscopy, no visible entity was present.

The electron microscope was developed in the 1930s-1940s, and it was possible to see viruses for the first time. Many phages isolated from the environment are members of the order Caudovirales, and consist of a head or capsid, and a tail. Within the head is the phage's double-stranded DNA, the genetic material that contains the instructions for making more identical phages. The tip of the tail and the tail fibers recognize and bind to the outside of the bacterial host. During infection, the DNA passes from the head and into the cell, while the protein components of the particle remain outside. The Caudovirales are split into three groups based on the morphology of the tail: the Myoviridae, which have contractile-tails, the Podoviridae, which have short stubby little tails, and the Siphoviridae, which have long flexible non-contractile tails. There are other types of phage virion morphologies, but the Caudovirales predominate.

Bacteriophage Life-cycles: Lytic growth

During lytic growth, the phage binds to the cell and then injects its DNA. Host machinery is used to express phage proteins needed early in the infection process. The phage DNA is replicated many times. Phage structural proteins are then synthesized late during infection, and self-assemble into capsid and tails. During DNA packaging, the DNA is stuffed into the heads, and then the tails are attached to finish the mature virion particle. In the final step, the cell undergoes lysis and releases 50-100 newly formed phage progeny viruses, which are capable of repeating the entire cycle with a new host cell.

Bacteriophage life-cycles: Lysogeny

When a temperate phage infects a host, it may undergo lytic growth as described above, or it may undergo a process known as lysogeny. During lysogeny, the lytic genes are switched off and the phage DNA stabilizes itself within the host cell for propagation during future cell divisions. Frequently this occurs through integration of the

phage genome into the host genome. Lysogens can be stable through many generations, or they can be induced to undergo lytic growth, either spontaneously or through DNA damage by UV or another agent. Phages that can only undergo the lytic cycle generally make clear plaques because all of the bacteria within the plaque have been killed. Temperate phages, on the other hand, make turbid or cloudy plaques. Turbid plaques are the result of some bacteria in the plaque being killed by lytic phage, while other bacteria, in which lysogeny has been established, are able to continue to grow even though they are surrounded by phage. This ability of lysogens to resist further infection by the same, or closely related, phage is known as immunity to super-infection. Lysogens can be isolated from turbid plaques, and used to make lawns on agar plates; phages within the same immunity group as the lysogen phage will not be able to make plaques on the lysogen lawn.

Integration of the phage genome into the host genome typically occurs through site-specific recombination and is catalyzed by a phage-encoded enzyme called integrase (Int). Recombination occurs between two sites: the attP (or phage attachment) site within the phage genome, and the attB (or bacterial attachment) site within the host genome. Integrated phage genomes are called “prophages”, and are flanked by the attL and attR (left attachment and right attachment) sites. Integration may require host-encoded proteins as well, such as IHF (integration host factor). Excision of the prophage from the host genome requires integrase, IHF, and another phage-encoded protein called excise (Xis), which dictates the directionality of the integrase-mediated recombination event.

Phages within the Environment: Numerous and Dynamic

The development of DNA-binding fluorescent stains and advances in epi-fluorescent microscopy made it possible to enumerate viral particles in environmental samples by filtering a sample, such as seawater, treating with stain, and counting viral particles in an epi-fluorescent light microscope. Such experiments have made it clear that the viral population is vast. There are 106-107 viruses/ml in seawater and about 10 viral particles for every bacterium in all environmental samples (oceanic or terrestrial). Using these observations, it is estimated that the entire biosphere contains 1031 viruses; the majority of which are bacteriophages. This suggests that there are more bacteriophages in the biosphere than all other biological entities taken together.

Not only are phages abundant but the population is dynamic. Ecological studies suggest there are 1023 infections of bacteria by phage every second, globally. This rate of

infection implies that the entire global phage population turns over every four or five days. At the time of this lecture, 650 phage genomes have been completely sequenced, which allows us to examine how similar or different they are from each other.

Summary

Bacteriophages are viruses that can infect bacteria. The most commonly found isolates belong to the order Caudovirales and have a tail and a double-stranded DNA filled head. Upon infection, many phages undergo lytic growth, in which the host cell becomes a virus manufacturing and assembly plant, the final result of which is the lysis of the cell and release of new progeny phages. Some phages can also stably incorporate their genomes into the host cell for replication and transmission to daughter cells after cell division; such cells are called lysogens. Lysogens can be induced, at which point the phage can undergo the lytic cycle.

Phages represent the majority of all biological entities in the biosphere. They are a dynamic population that has been evolving for a long time, and they provide the largest unexplored reservoir of new genetic information.

3. Recommended Reading

1. Microbiology with Diseases by Body System, 3rd edition. Robert Bauman, Ch. 13.
2. Chapter 7. Molecular Genetics of Bacteria 4th edition. Snyder et al. ASM Press. ISBN: 978-1-55581-627-8
3. Adv Virus Res. 1998;51:135-201.
4. Tailed bacteriophages: the order caudovirales.
5. Ackermann HW

4. Review Questions

1. The killing agent of D'Herelle and Twort was so mysterious because:

a. Filtered samples appeared not to contain anything of biological origin when examined by light microscopy
b. Small amounts of the agent led to complete clearing of liquid bacteria cultures
c. Serial dilution of the agent lead to small localized zones of bacterial death when mixed with cells and plated on a lawn
d. all of the above

2. During lytic growth the correct order of events is:
    a. adsorption, capsid assembly, lysis, DNA replication, DNA packaging, late protein synthesis
    b. adsorption, DNA replication, late protein synthesis, capsid assembly, DNA packaging, lysis
    c. DNA replication, lysis, DNA packaging, capsid assembly, adsorption, late protein synthesis
    d. lysis, DNA packaging, DNA replication, adsorption, capsid assembly, late protein synthesis
    e. none of the above

3. Integration of the prophage into the host genome requires (choose as many as you like):

4. attP, attB, attL, attR, integrase, IHF, Xis, RNA polymerase, lysis, DNA replication, infection

5. What techniques were used to supply the evidence that suggests that bacteriophages are the most numerous entities in the biosphere?

6. Why are plaques from temperate phage turbid?

5. Answers to Review Questions

1. D

2. B

3. attP, attB, integrase, IHF, infection

4. DNA staining and epifluorescence microscopy

5. The presence of lysogens growing within the plaque makes the plaque turbid.

6. Discussion Questions

1. What does the generation of clear plaques suggest about a phage's life cycle?
2. What does it mean when two different phages belong to the same immunity group?
3. How big is 1031? What does this mean about the global population of bacteriophages in relation to other living organisms?
4. Why do we think there are 1023 bacteriophage infections/second globally? What does this means about how fast the population of bacteriophages turns over?

7. Answers to Discussion Questions

1. It is likely that the phage is not able to form lysogens on this particular host, and it is therefore only undergoing the lytic cycle.
2. A lysogen generated by one phage will be resistant to super-infection by the other.
3. Really really big. If the average phage is 125nm long, all those phages laid end to end would still be 1.3x108 light years long (or about 1000x longer than the diameter of the Milky Way!) Because there are simply so many phages, there are more phages than all of the rest of the organisms on the planet added together.
4. Once we know what the global populations of bacteria and bacteriophage are (1030 and 1031), it is possible to determine how often they bump into each other (frequently), and how often those contacts result in a productive infection (less-frequently). However, since our starting numbers are so enormous, we can extrapolate that the number productive infections is 1023 per second. This means that the entire global population of phage turns over every few days.

8. Explain or Teach These Concepts to a Friend

1. Explain how phages grow lytically.
2. Explain the difference between integration and excision.

3. Explain how the presence of bacteriophage in the “killing agent” explains the observations of D’Herelle and Twort and an antibiotic chemical does not.
4. Explain why scientists believe the global population of phages is so large and so dynamic.

9. Research the Literature on Your Own

1. Can you find any human pathogens that are actually lysogens, with prophage-encoded human toxins (Hint: look up bacteriophage CTX phi)?
2. Do you know of any key molecular biology concepts that were experimentally determined using bacteriophage? (Hint: look up Hershey and Chase)
3. Is there any evidence that demonstrates an evolutionary relationship between bacteriophage and animal viruses? (Hint: look up Herpes virus and phage HK97)