BACTERIOPHAGE

This semester we have examined fungi infecting bread and fungi and bacteria infecting fruits and vegetables. We will now focus on viruses that infect bacteria. These viruses are called bacteriophage, or phage for short. Bacteriophage were discovered nearly a century ago as particles that lyse bacteria. Many fundamental discoveries in biology have been made in studies of bacteriophage, including regulation of gene expression, nonsense codons stopping translation, and mechanisms of natural selection. However, it has only been in the last 15 years that scientists have come to appreciate the vast role of bacteriophage in our biosphere. Current estimates place the number of phage on earth at 10^{31} or 10 billion trillion trillion, roughly 10 phage per microbe! In terms of pure numbers, this makes phage by far the most numerous life form on the planet. There are more phage on earth than stars in the universe. Stacked end-to-end, all the phage on earth would make a column 100 million light years long, 1000 times the diameter of the Milky Way galaxy. Phage have profound effects that we are only beginning to understand on the ecology and health of every environment on earth from the oceans to the human body.

Like all viruses, bacteriophage cannot reproduce on their own and must infect a host cell to grow and reproduce. They bind to an appropriate host, inject their genomes, and use host machinery, such as ribosomes, to synthesize new phage particles. Bacteriophage come in two basic types- lytic and temperate. Lytic bacteriophage lyse (burst open) the host bacterium, once mature phage particles accumulate in the cell. The phage that are released then go on to infect nearby cells. Temperate bacteriophage infect bacteria like lytic phage, but occasionally instead of lysing the bacteria they integrate their genomes in the host chromosome and essentially become part of the host genome. If their host bacterium is stressed, the temperate phage can excise from the chromosome, make new phage particles, and lyse their host. We will focus on lytic phages in this course.

To get acquainted with bacteriophage, we will start our work with a classical assay used to isolate and titer (count) bacteriophage. In the following lab units, we will use phage to explore the role of selection and mutation in evolution and then we will investigate bacteriophage carried on the human body.

Isolating and Titering Bacteriophage

There are two principle methods of growing lytic phage, both based on the ability of phage to kill the host bacterial cell. One method introduces phage into a liquid bacterial culture. After a period of incubation, the phage lyse all the bacteria in the broth culture, resulting in a clearing of the growth medium. This method was used to make the phage stock for this lab procedure and is used primarily to grow large amounts of bacteriophage. To isolate particular phage and to determine the number of phage in a phage preparation, we use a plaque assay. In this assay, phage and bacteria are mixed with a few milliliters of soft agar. This soft agar mixture is poured over a hard agar base. Bacteria will grow in the soft agar making it cloudy. After a period of incubation, zones of

clearing, known as plaques, will form in bacterial lawn in the top agar due to phage activity. Each plaque derives from a single phage particle in the original sample that infected a single cell. Hundreds of phage can be released from the infected cell. These phage then invade neighboring cells, ultimately resulting in lysis of all the bacteria in the vicinity of the initially infected cell.

Plaque assay procedure:

1. Make a serial dilution series out to the 10^{-8} dilution with your phage stock, as described in the serial dilution protocol.
2. To get an accurate phage count, you want to have 30-300 well-separated plaques on a plate. You should see this for the 10^{-6}, 10^{-7} or 10^{-8} dilutions of the phage you are using today.
3. Remove a soft agar tube from the 50 °C water bath, add 0.1 ml of bacteria to the soft agar, followed by 0.1 ml of diluted phage.
4. Roll the agar tube between your palms to mix for 2 or 3 seconds, and quickly pour the contents onto the agar surface of the hard agar base plate.
5. Quickly and gently rock the plate to disperse soft agar over the entire surface of the base plate agar.
6. Allow soft agar to harden. Incubate inverted at 35 °C. The appearance of plaques will depend on the phage, but should be visible in about 6 – 24 hours. If over-incubated, plaques can continue to enlarge. Given sufficient numbers of plaques, this may result in confluent lysis of all the bacteria in the soft agar.

*It is important to work quickly when doing steps 3 - 5 or the soft agar will harden before you get it spread on the plate.

Serial Dilution Technique :

Often bacterial or bacteriophage cultures are highly concentrated, with 10^9 bacterial cells per ml or 10^{10} bacteriophage per ml. Simply plating a 100 µL aliquot of these cultures would result in way too many colonies or plaques to count (10^8 colonies or 10^9 plaques), because each colony and plaque is formed from a single bacterium or bacteriophage respectively. Serial dilution is useful for obtaining countable numbers of bacterial colonies or bacteriophage plaques. The protocol described here is for serial 1:10 dilutions and can be modified for any dilution series desired.

Procedure:

1) Label tubes 10^{-1}, 10^{-2} etc. through 10^{-8}, or carry out to whatever dilution is appropriate.
2) Label destination plates of appropriate solid medium with the dilution and identifying information. Because your goal is to obtain 30-300 well isolated plaques on a plate, generally only the 10^{-6} through 10^{-8} dilutions are plated; however, the choice of dilutions to plate will vary.
3) Pipet 0.9 ml of sterile water or broth into the appropriate number of tubes labeled in step 1.

4) Using a P200 micropipettor, transfer 100 µL of a 1:100 dilution to the 0.9 ml diluent in the tube labeled 10^{-1}, mix well by tapping the tube with your finger.
5) Using a new tip, transfer 100 µL ml of the 10^{-1} dilution to the 0.9 ml diluent in the tube labeled 10^{-2}. Mix well. This makes a 10^{-2} dilution of the original culture.
6) Continue sequentially in this manner until you have carried the dilution to an appropriate end 10^{-8} in this case. Remember to change the pipette tip and mix the diluted phage in between each dilution
7) Label your plates with the appropriate dilution, your name, and the date.
8) Following the protocol for the plaque assay, using the appropriate dilutions.

References:
http://www.bionumbers.hms.harvard.edu
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Zimmer C. (2011) A Planet of Viruses, University of Chicago Press, Chicago IL.