ENVR 421 Laboratory #4: Plaque assay for bacteriophages

Introduction

Bacteriophages are viruses that infect bacteria. Although structurally similar to some human viruses, they belong to separate virus families and do not cross species barriers. In environmental microbiology, we are interested in bacteriophages for two main reasons. First, because of their structural similarities to human viruses, observing their occurrence and survival in the environment can give us some ideas about how human viruses behave under environmental conditions. Second, some bacteriophages occur naturally in the human and animal intestinal tract and are excreted in feces. Thus, phages can be indicators of fecal contamination in the environment. Phages are also useful for mastering techniques for working with viruses, since they are simpler to work with and faster-growing than most human viruses.

Purpose

The purpose of this laboratory exercise is to familiarize you with one of the types of plaque assay for viruses, the double agar layer (DAL) assay. The DAL plaque assay allows us to count the number of viruses in a sample. As with E. coli in the spread plate technique, we will make a dilution series, which ensures that the viruses placed on plates are few enough to be counted easily.

Principle

The plaque assay is a direct count method for assaying virus infectivity. It is based on the ability of the virus to infect and kill its specific host bacterium. In the assay, a mixture of warm liquefied agar (“top agar”), virus, and host bacterium is made. Viruses in the mixture attach to cells and begin the process of infection. This mixture is poured onto the surface of a solid agar plate (“bottom agar”). The top agar will then solidify, creating a layer of agar with bacteria growing in it. The bacteria will grow throughout the top agar layer, creating a continuous layer of bacteria called a lawn. Viruses are attached to cells in this lawn, and they will infect the bacteria as the plates incubate. As the bacteria grow, the viruses begin to spread from the cells they initially infected to the surrounding cells, but the agar limits how far they can spread. This results in small patches of dead virus infected cells in the lawn, with each patch resulting from a single virus. When you look at
the plate against a light, the infected killed cells are visible as clear spots, or plaques, surrounded an opaque lawn of live bacteria. These plaques are counted and used to calculate the amount of virus present in a sample.

This is a generic plaque assay protocol that will work for bacteriophages that have the ability to form plaques on a host. Specific bacteriophages need specific host bacteria. In our lab, the most common bacteriophages are male specific coliphages (MS2), somatic coliphages, and PRD-1. Male specific coliphages are grown with host *E. coli* Famp, which will NOT grow somatic coliphages. Somatic coliphages are grown with host *E. coli* CN-13, which will NOT grow male specific coliphages. Host *E. coli* C3000 will support the growth of both somatic and male specific coliphages, but will not allow you to differentiate between the two. Hosts *E. coli* Famp and CN-13 carry antibiotic resistance traits on plasmids. If these hosts are used, antibiotics can be added to the media to suppress the growth of competing bacteria.

*Preparation of bacterial host cultures*

In order to do a successful plaque assay, the bacteria must be in the exponential phase of growth. This is prepared by growing two separate bacterial cultures. The first culture (the overnight) is allowed to grow for 18-24 hours, resulting in very high numbers of bacteria in the culture. Some of this culture is used to start another culture (the log phase), which will be allowed to grow for 2-4 hours. This culture will enter the exponential phase during the 2-4 hour time period. When it does, it is suitable for use in the plaque assay. When the culture becomes cloudy, the OD$_{520}$ is measured using the spectrophotometer. The OD reading should be somewhere between 0.3 and 1. Once it reaches this level, it is ready for use.

*Making top agar:*

The plaque assay uses tryptic soy broth with a solidifying agent (Bacto agar). Top agar consists of 30g tryptic soy broth and 7.5g of Bacto agar per liter of top agar. This makes a top agar that has HALF as much agar as standard tryptic soy agar plates. (If the agar concentration is too high, it will interfere with plaque formation. This is why you cannot use standard TSA as top agar). When poured on a plate, this will create a semisolid layer of media that will both feed the host bacteria and allow visible plaques to form.
Materials needed:

- 10 dilution tubes of PBS
- 200µL pipette tips (preferably aerosol barrier)
- 1000µL pipette tips (preferably aerosol barrier)
- vortexer
- 1 tube *E. coli* log phase host
- 1 tube MS2 phage stock
- 11 top agar tubes
- 11 bottom agar plates

Protocol

Preparation of the host culture for this lab will be done for you by the TA.

1. Make 10 dilutions of your virus stock or sample by adding 100 µL stock to 900µL PBS. Each plate can be inoculated with up to 1 mL of sample. Dilutions can be made in sterile water, PBS, TSB, or other diluents; we will use PBS
2. Label your bottom agar plates and put them out on the bench
3. Work with only a few samples at a time, so the top agar does not harden while you are working. Remove 4 tubes at a time from the waterbath and place in a rack in front of you.
4. Test against your wrist to ensure agar is not too hot.
5. Add 1mL of host bacteria to each tube using a large pipette
6. Add the entire 1 mL of sample from the lowest dilution tube (one with the least virus) to a top agar tube using a micropipettor
7. Pour the contents of the tube into a plate.
8. Repeat with the rest of your dilutions
9. Add host to the last top agar tube
10. Pour into a plate. This is the negative control
11. Once plates solidify, invert and incubate at 37°C 16-24 hours.
12. Count plaques using a light box.