Post-Activity Assessment

Direction: **Answer the following questions from the lab activity.**

1. Is there a difference in the four (4) plaques formed in Plate 1? If yes, compare the difference of the four (4) plaques in plate 1. Use qualitative and quantitative observations.

   _____
   _____
   _____
   _____

2. Is there a difference in the four (4) zones of inhibition in Plate 2? If yes, compare the difference. Use qualitative and quantitative observations.

   _____
   _____
   _____
   _____

3. What are your observations in Plates 3 and 4? Describe the importance of these two (2) plates in the experimentation.

   _____
   _____
   _____
   _____

4. Which plates are the control and experimental set-ups?

   _____

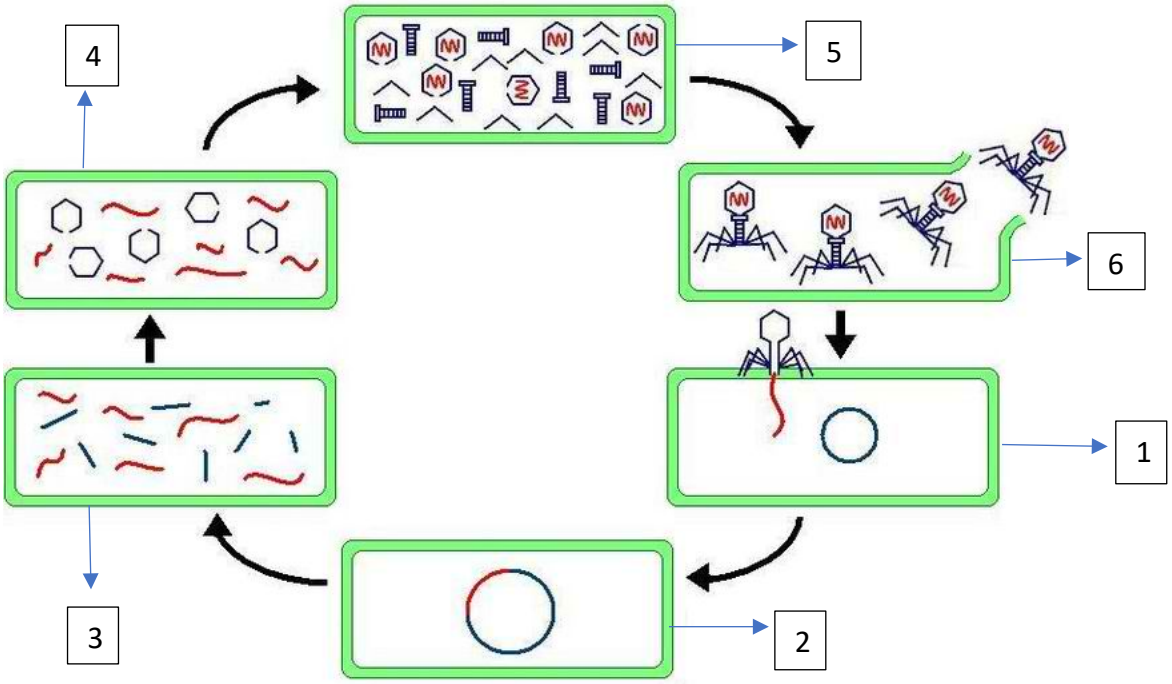
5. Which one do you think works better in controlling or destroying bacteria, the phage or antibiotic?

   _____
   _____
   _____
   _____

Activity Embedded Assessment

THE LYTIC CYCLE

Describe the events that happen in each stage of the Lytic Cycle



1. _____
   _____
2. _____
   _____
3. _____
   _____
   _____
4. _____
   _____
   _____
5. _____
   _____
   _____
6. _____
   _____
   _____

Visualizing Lytic Infection

Subject Area(s) : Biology, Life Science

Activity Title. : Visualizing Viral Plaques Using Drop Spot Test Method

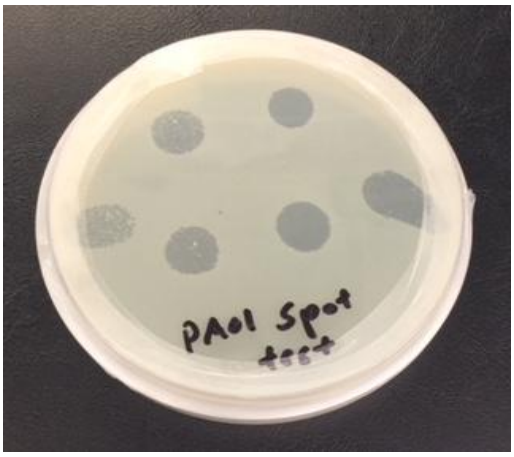


Figure 1. Viral plaques from PEL1 polyvalent phage and *Pseudomonas aeruginosa* bacteria

Grade Level	**9-12**
Activity Dependency	**None**
Time Required	**60 minutes**
Time Required Note	**30 mins for set-up and another 30 mins for observation after 24 hours.**
Group Size	**3 - 4**
Expendable Cost/Group	**US $3**

Summary

Students learn about how viruses such as bacteriophages infect bacteria through the lytic cycle. Students will visualize the infection through the formation of viral plaques which are clear regions in the petri plates of cell destruction in the bacterial culture. Students also determine the polyvalency of a phage through testing with different bacterial hosts.

Engineering Connection

Civil, environmental, and chemical engineers use bacteriophages in addressing water-related issues such as bacteria-induced corrosion in pipes. These viruses are used to target

problematic bacteria in biofilm instead of reliance to antibiotics and harsh chemicals. Phage-based mitigation in wastewater treatment has been one of the areas of interest under the Nano-Enabled Water Treatment (NEWT) project.

Engineering Category = #1 Relating science and/or math concept(s) to engineering.

Keywords
Bacteriophage, bacterial lawn, bacterial host, biofilm, culture plate, lytic infection, nutrient agar, phage,, polyvalence, soft agar, viral plaque, virus, water treatment, zone of inhibition

Educational Standards
Texas Essential Knowledge and Skills for Science - Biology : §112.34
4C. compare the structures of viruses to cells, describe viral reproduction, and describe the role of viruses in causing diseases such as human immunodeficiency virus (HIV) and influenza.

International Technology and Engineering Educators Association - Technology & Society and The Designed World

Standard 5: Students will develop an understanding of the effects of technology on the environment.

Standard 14: Students will develop an understanding of and be able to select and use medical technologies.

NGSS Standards: Science - Planning and Carrying Out Investigations

Plan and conduct an investigation individually and collaboratively to produce data to serve as the basis for evidence, and in the design: decide on types, how much, and accuracy of data needed to produce reliable measurements and consider limitations on the precision of the data (e.g., number of trials, cost, risk, time), and refine the design accordingly. (HS-LS1-3)

Pre-Requisite Knowledge
1. Students should have a basic understanding on the different types of viruses, their viral structure and respective hosts.
2. Students should understand that a virus needs a host to replicate.

Learning Objectives
After this activity, students should be able to:
a) Describe how a phage infects a host cell.
b) Compare the infectivity between a phage and antibiotic.
c) Explain why antibiotics work on bacteria but not on viruses.

Materials List

Each group needs:

- 4 petri plates with 20 ml of nutrient agar per plate
- 5 ml of soft nutrient agar per plate
- 4 drops of 10 uL *E. coli* phage per plate
- 50 uL of *E. coli* bacteria
- antibiotic discs
- pipettors or disposable pipettes
- microcentrifuge tubes
- 5-mL pipettes or small graduated cylinder
- parafilm or transparent tape
- 70% isopropyl alcohol or 10% bleach solution.
- 2 10-ml Eppendorf tubes
- gloves and apron
- hot plates or water bath
- 250-mL beakers

To share with the entire class:

- Nutrient agar and soft agar (Note to teacher: The teacher can prepare a stock for the whole class. Use the dilution specified in the nutrient agar container. To prepare for the soft agar, double the amount of distilled water required in the container. Students can measure the amount required for each culture plate from the stock and students prepare the petri plates themselves).
- Phage stock (Note to teacher: To avoid contamination, give each group a microcentrifuge tube with enough amount of phage needed for the test instead of passing around one tube of phage stock to all the groups.
- Bacterial host stock (Note to teacher: Same as phage stock. Phage and bacteria can be purchased from suppliers such as Carolina Biological or Sargent Welch, etc)

Introduction / Motivation

(Have a powerpoint ready to show the following questions with corresponding images of the condition or pathogen.)

What does the doctor usually prescribe you to take when you have an ear infection, or a toothache, or a strep throat? Why is it that antibiotic works on these conditions? What about when you have a cold or flu? Can you take antibiotics as well? if not, why?

For a long period of time, humans have been dependent on antibiotics to treat or solve bacteria-related problems. It is not only used to treat human diseases but also in raising animals such as pigs and chicken. Environmentally, one of the main causes of corrosion in water pipes is biological which is mainly an aggregate of different types of bacteria forming a biofilm.

Antibiotics can be used as well to destroy these problematic bacteria. However, the excessive use of antibiotics to address bacteria-related issues lead to the evolution of some bacterial species to develop resistance against certain antibiotics. It became a challenge for humans to quickly design new antibiotics to destroy these newly evolved bacteria.

Viruses such as bacteriophages (or phages) are known to infect bacterial cells. Most of the time, a virus is specific to its host. Thus, one phage only infects one type of bacterial host. However, studies revealed that there are phages known to be polyvalent, that is, one phage can infect multiple types of bacterial hosts. Researchers have been investigating these polyvalent phages in terms of its possible use to solve bacteria-related problems in the environment.

In this experiment, students observe how a virus infects a bacterial host. If antibiotics inhibit the growth of bacteria forming a zone of inhibition, viruses infects bacteria forming viral plaques. The drop spot test method is a qualitative test to show the infectivity of a virus to its host.

Vocabulary / Definitions

Word	Definition
Bacteriophage	also known as "phage." It is a virus that destroys bacteria (host) only.
Bacterial host	refers to any type or species of bacteria. e.g. *E. coli, P. aeruginosa*
Bacterial lawn	a mat or cover of bacteria throughout the surface of the culture media.
Biofilm	is an aggregate of one or more species of bacteria that grow on different type of surfaces.
Culture plate	also called 'petri plate or petri dish." It is used to grow microorganisms such as bacteria.
Lytic infection	also refers to 'lytic cycle', is the infection of a bacterium by a bacteriophage which results to the destruction of the bacterium and release of new bacteriophages.
Nutrient agar	a growing medium enriched with nutrients for microorganisms such as bacteria. It is used as the base layer of the culture medium.
Polyvalence	is the ability of one phage to infect multiple bacterial hosts
Soft agar	a nutrient agar that is prepared by doubling the amount of water required in the preparation. It is used as the 2nd layer (top) of the culture medium for bacterial lawn.
Zone of inhibition	a clear, circular zone in the culture where bacteria stopped from growing using the antibiotic discs.

Procedure

Background

In this activity, students visualize the effects of a bacteriophage or phage infecting a bacterial host using the Drop Spot Test method. Viral infection is manifested through plaque formation on the top layer of the culture medium which is the soft agar. One (1) type of phage is used in the experiment to infect a bacterial host. Antibiotic discs are also used on these bacteria and results are compared between the effects of antibiotic and phage.

Before the Activity : Teacher Preparation

- *Preparation of the nutrient agar*

Use the instructions written on the container of the nutrient agar (Ex: dissolve 23 g of agar into 1000 mL of dH_2O). Autoclaving is highly recommended but can proceed without it as long as the aseptic techniques are observed in conducting the lab. Prepare the nutrient agar for the entire class.

- *Preparation of the soft agar*

Follow the instruction written on the container of the nutrient agar but double the amount of dH_2O. Prepare the soft agar for the entire class.

- *Preparation of the bacterial host*

Bacterial cultures from suppliers may require subculturing using the broth provided before the start of the lab. Allow the bacteria to grow in the broth at least 48 hours at 37°C or 72 hours at room temperature (23°C).

Day 1

1. Divide the class into three (3) per group.
2. Ask them to wear their gloves and apron for the lab activity.
3. Instruct the class to disinfect their working station with 70% isopropyl alcohol or 10% bleach solution..
4. Ask one (1) person from each group to collect the materials. Be sure to remind the students not to open the petri plates and microcentrifuge tubes to prevent contamination. (Option: Microcentrifuge tubes containing the phage and bacteria can be distributed later as needed during the activity.)
5. Label four (4) petri plates as follows;
   Petri Plate 1 : *E. coli* + phage
   Petri Plate 2: *E.coli* + antibiotic
   Petri Plate 3: + Control (only *E. coli* bacteria)
   Petri Plate 4: - Control (only nutrient and soft agar, no bacteria)
6. Guide the class to the whole process of the Drop Spot Test.
   a. Transfer 20 ml of nutrient agar to 4 petri plates. Slowly swirl the agar to evenly distribute it on the plate. Close the petri plates and wait for nutrient agar to solidify. The nutrient agar serves as the base layer of the culture media.
   b. Once the agar solidifies or is fixed, turn the plates upside down to prevent condensation.
   c. Measure 5 ml of soft agar and transfer it to an Eppendorf tube or any clean small tube. (Note: Keep the Eppendorf tube with the soft agar in a water bath at around 46°C so it will not solidify since it solidifies fast at room temperature.)
   d. Add 50 uL of *E.coli* into the Eppendorf tube with the soft agar. Swirl to mix.
   e. Immediately, pour the mixture of soft agar and *E. coli* into petri plate 1. Soft agar is the top layer of the culture media. Swirl the mixture to evenly distribute the bacteria on the plate. Wait for the soft agar to solidify and then close the petri plate.
   f. To perform the Drop Spot Test, pipette 10 ul of *E.coli* phage and drop it on the four (4) corners of petri plate 1. Each drop serves as a replicate. Wait for the phage to dry on the soft agar, close the plate and invert it to avoid condensation. Seal the petri plate with a parafilm or masking tape to keep the moisture inside the culture media.

g. Incubate the petri plates at 37°C for 24 hours, if incubator is available. The use of incubator is highly recommended but can be done without it, however longer time may be needed to allow bacteria to grow and create a lawn on the soft agar layer.
h. For the antibiotic test, repeat steps c to g for plate 2, but replace the 10ul of phage with a disc of antibiotic.
i. Repeat steps c to e for Plate 3.
j. Repeat steps c and d for Plate 4.

Day 2

1. Observe the plaques formed in petri plate 1.
2. Observe the zones of inhibition in petri plate 2.
3. Compare the difference.
4. Observe what happened in petri plates 3 and 4. These two plates will validate the results of petri plates for contamination.

Safety Issues

- Ensure that students wear gloves at all times during the experimentation.
- Proper disposal of culture media with microorganisms should be followed at the end of the activity.

Troubleshooting Tips

If plaques are not formed, check the bacterial culture. There should be enough bacteria growing throughout the top layer (soft agar) to ensure lytic infection of phages.

Assessment

Pre-Activity Assessment

Review: Compare and discuss bacteria and viruses using different features such a ; size, structure, genetic material, metabolism, pathogenicity, treatment, and reproduction.

Activity Embedded Assessment

Explain: Discuss the lytic infection and make students complete the Lytic Cycle worksheet during the discussion.

Post-Activity Assessment

Questions: Students answer the questions on the post-activity worksheet based on the results of the experimentation.

Activity Extensions

Use the phage from the supplier to infect a different type of bacteria to test for its polyvalency.

References
P. Yu, et.al, Appl environ Microbiol, 2016, 82, 808-815.
Devin Wood. Virus Prezi (Lytic cycle flowchart), 2014

Contributors
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