PreMiEr
Precision Microbiome Engineering
NSF Engineering Research Center

Assessing Microbial Reproduction and Viral Lysis

Exploring how phage affects the growth of *E. coli* bacteria

Overview

Students are able to explore the process of asexual reproduction of microorganisms found within their classroom/school environment and analyze the efficiency of microbial growth through observation of isolated colonies. Students will implement the methods of swabbing microbial samples and agar plating to yield results. With completion of this lesson, students will be able to not only observe the reproductive processes undergone by microorganisms, but also deduce reasoning, using observable evidence, as to how phages and/or antibiotics affect the reproductive efficiency of their samples.

Key Search Words

Biology (Science)
9-12th grades
Asexual Reproduction - Binary Fission - Bacteria - Unicellular Organisms - Antibiotic - Phages
Hands-on Investigation

Learning Objectives

- To observe and describe bacterial reproduction by growing microbes from swab samples and *E. coli* cultures.
- To use bacterial colony growth as a model to understand how microorganisms reproduce through cell division.
- To explain how bacteriophages reduce bacterial growth through infection and lysis.**
- To interpret plaque assay results as evidence of viral impact on microbial populations and possible implications for bacterial resistance.

Curriculum Alignment

- **LS.Bio.2.1** Use models to illustrate how cellular division results in the reproduction, growth, and repair of organisms. (North Carolina Department of Public Instruction, 2024, p. 2)
- **LS.Bio.9.1** Analyze and interpret data to summarize how various factors such as geographic isolation, pesticide resistance, antibiotic resistance can influence natural selection. (North Carolina Department of Public Instruction, 2024, p. 4)
    - *See “author comments” below*

Classroom time required

- **Two Consecutive Class Periods (Block - 1 hr 30 min)**
    - Day 1 - Swabbing, enrichment, and plating of bacteria and phages
        - Teacher guided discussion / Review of lab instructions - **15-20 min**
        - Collection of swab samples - **15 min**
        - Enrichment of samples - **20 min**
        - Plating of samples - **10-15 min**
        - Student lab worksheet - **15 min**
        - DFA (Daily formative assessment) - **5 min**

    - Day 2 - Observation and analysis of bacterial colonies
        - Small group review of samples / complete student lab worksheet - **20 min**
        - Class discussion - **10-15 min**
        - Student reflection worksheet - **15-20 min**
        - Small group poster creation - **30 min**
        - DFA (Daily formative assessment) - **5 min**

- **Three Consecutive Class Periods (Traditional - 50 min)**
    - Day 1 - Swabbing and enrichment
        - Teacher guided discussion / Review of lab instructions - **15-20 min**
        - Collection and Enrichment of swab samples - **20 min**
        - DFA (Daily formative assessment) - **10 min**
    - Day 2 - Plating of samples
        - Review of lab instructions - **5-7 min**
        - Plating of samples and phage - **15 min**
        - Student lab worksheet - **15-20 min**
        - DFA (Daily formative assessment) - **10 min**
    - Day 3 - Observation and analysis
        - Small group review of samples / complete student lab worksheet - **15 min**
        - Class discussion - **10 min**
        - Student reflection worksheet - **15-20 min**
        - DFA (Daily formative assessment) - **5 min**

***Upon teacher discretion, lesson can be extended to fourth day to include poster presentation as a form of assessment or project grade.**

Materials & Technology

Lab Materials

- Prepared agar plates
- Micropipettes / pipette tips
- Microtubules / test tubes
- Buffer
- LB Broth
- Sterile swabs or Q-tips
- Tubes (15 mL or 50 mL)
- Erlenmeyer flask
- Sterile cell spreader
- E. coli host culture (K12 strain or equivalent)
- Incubator (or warm, contained area)
- Top Agar
- Phages (T4 or T7) and/or appropriate antibiotic (I.e. ampicillin)

Safety Materials

- Gloves
- Safety goggles
- Lab apron

Resource Materials

- Asexual reproduction powerpoint
- Lab worksheet
- Reflection worksheet
- Poster presentation rubric

*** Personal powerpoint use can be at teacher's own discretion to best fit their lesson**

Safety
This lab will require students to collect swab samples of unknown microorganisms, manipulate their environment to enhance reproduction, and closely observe the growing bacterial colonies. Students should, at all times, handle materials while wearing gloves, lab apron, and safety goggles. Agar plates, pipette tips, and other material should be discarded in appropriate waste containers.

Teacher Preparation for Activity
Before the day of class, the teacher should familiarize themselves with any powerpoint or materials on asexual reproduction and unicellular organisms (prokaryote vs eukaryote) for student recall of information during class discussion and introduction of the lab. A tip for preparation is to incorporate bellringer activity that focuses on recall of information.

Teacher should ensure that students are familiar with the processes they will be implementing during lab; this includes— pipetting, proper waste disposal and handling, and laboratory safety. Teacher could consider a “mock” pipetting activity days prior to activity to ensure students are appropriately carrying out their technique.

Teacher should also prepare materials for lab by ensuring materials are in place and every student and/or small group is accounted for— this includes materials necessary for investigation, handouts, student reflection sheets, etc. Consider incorporating small group stations to encourage collaboration and promote efficiency for teacher/student interaction throughout lesson. Teacher should also account for the time spent at each location within the classroom and/or school in which they will allow students to collect swab samples.

Student Preparation for Activity
Before engaging in this activity, students should have prior knowledge related to the topics of asexual reproduction and unicellular organism / microorganism characteristics. While it is not required, teachers can assign students pre-reading and/or homework on said topics to refresh their memory of the information they will be investigating. For instance, in previous days before introducing the activity, teachers can engage students in the concept of microorganisms by having them look at different types of cells under a microscope; this will be a refresher for them that the organisms they will explore in this activity are not naturally visible to the naked eye.

Procedure
DAY 1

- Teacher should begin lesson by facilitating class discussion and recap the topics of microorganisms (prokaryotic characteristics), asexual reproduction, and discuss what phages are and how they are being used medically as a way to control harmful bacteria. Within this introduction, teacher should reiterate the processes students will observe within this activity. Teacher should reiterate laboratory safety and emphasize the steps that will be taken to complete the task (including how much time will be designated for each part, locations for student choice of sample collection, hallway etiquette, etc.). Teacher should also review lab instructions with students, ensuring the understanding that you will be guiding them through each step and that they should not be working ahead.
- After review of lab instructions, teacher should assign materials necessary for swabbing to each student / small group, and walk with students through classroom and/or designated spots within school, monitoring the time spent at each location and reiterating to students that they have one choice of location for sample collection.
    - Teacher should handle a secure tube of buffer for student use of swabbing.
    - Teacher should ensure that once samples are collected, students are placing them in secure tubes to avoid contamination.
    - Teacher should ensure students are rotating swabs in area of sample collection.
- Once swabs are collected, teacher will guide students back to classroom stations to begin enrichment and plating processes. Teacher should walk students through preparing their E. Coli samples and E. Coli + phages mixture in appropriate tubules. Be sure to remind students of careful handing practices to keep agar plates and samples from being contaminated (i.e. opening agar plates using clamshell method)
    - Students will also need to label 3 agar plates— one for swab sample, another for E. coli control sample, and last for E. Coli + phages mixture. Teacher should actively monitor students, ensuring they are plating and smearing the appropriate samples on their respective plates, one at a time, using a streaking tool.
- Once plating is complete, students should place them in proper storage for incubation. After cleaning material, workspace, and appropriately disposing of waste materials, students should then begin to fill in lab worksheet in small groups.

DAY 2

- Teacher should review lab instructions with students, reiterating laboratory safety, the previous day's procedure, and things to look for when observing samples.
- Teacher should actively monitor students and engage to answer questions students may have as they make observations and engage in discussion. Students will complete lab worksheet, documenting their findings.
    - **Goals / Conclusions for discussion :**
        - To understand that microorganisms reproduces asexually from one unicellular organism.
        - To understand that asexual reproduction of microorganisms is a quick and simple process.
        - To understand that phages (similar to antibiotics) work to kill bacteria— extension of knowledge could be to introduce antibiotic resistance.

    - **Possible Prompt Questions :**
        - *"Through which process were these microorganisms able to reproduce? How is this similar or different to the way our [human] body cells reproduce?"*
        - *"How did we enrich the samples in this activity? Predict what the samples may have looked like if LB broth was not used. Would the microorganisms still reproduce?"*
        - *"Compare the E. coli control sample to the E. coli + phage mixture. How did the presence of phage affect the reproduction of these microorganisms?*
        - *"When comparing the phage mixture to the antibiotic mixture, which one seemed more effective? How do you know? Why do you think this is so?"* ***(appropriate only for experimental use of both phages and antibiotics)***

- After engaging in class discussion and completing reflection sheet, students will work in small groups on poster presentation, researching and explaining the impact of a desired phage/antibiotic on a specific microorganism. (***Can be extra credit and/or extension of reflection to meet time demands)***

Differentiation

Whole Class Modification

- Consider small groups for lab implementation

Advanced / Gifted Learner Modifications

- Extend student thinking using higher order questions on reflection sheet and/or poster presentation. Examples include asking students to consider other ways to enrich the environment of microorganisms, problem-solve ways in which their school/classrooms may be unintentionally serving as an "enriched" environment and how to combat it, or providing scenarios of different environments for students to predict which environments would be more suitable for bacterial growth and explain using reasoning.
- Having students serve as "team-leaders" in small groups

English Language Learners Modifications

- Provide students a modified lab and/or reflection worksheet that prompts for more visual results vs short answer responses
- Provide sentence starters for students to use when explaining results during class discussion

Assessment/Check for Understanding

Teacher will determine student understanding through reflection worksheet, daily DFA's of choice, and poster presentation— which students could present through verbal discourse or simply visual upon teacher discretion and time demands. Teacher should check for two key understandings:

1. Students understand that microorganisms reproduce asexually from a single cell, which is a simple and quick process.
2. Students understand that phages (similar to antibiotics) work to kill bacteria.

If teacher chooses to assess using poster presentation, these understandings should be expressed through the use of vocabulary terms such as: asexual reproduction, binary fission, prokaryotic organisms, unicellular, phages / antibiotics. Rubric is attached for assessment criteria.

Required resources

See attachments

- Assessing Microbial Reproduction and Viral Lysis Protocol
- Exploring Bacteriophages Powerpoint

Supplemental resources

See attachments

- Phage Discovery: Scientific References and Laboratory Protocols
- Optional Phage Isolation Protocol (extension of practice)
- "How to Kill a Superbug" Case study (Jeffers-Francis Lab)

Author comments

Please note that while viruses (phages) are not explicitly stated within the NC high school Biology curriculum, the phage experiment is designed for students to experience how there are agents produced, medically, in efforts to kill microbial organisms, such as bacteria. While T4 and T7 phages are emphasized in this lesson (a virus found to be effective in killing specifically *E. coli),* teachers may modify the lesson with the use of appropriate antibiotics— ensuring that the chosen antibiotic targets the respective bacteria students will be observing (i.e antibiotic "ampicillin" targets *E. coli*). Upon teacher discretion, this experiment can be completed with the use of just phages, just antibiotics, or with both phages and antibiotics to compare effectiveness (i.e. split class).

I incorporated the phage (antibiotic) experiments as a way of introducing antibiotic resistance, which is included in **LS.BIO.9.1** *("Analyze and interpret data to summarize how…antibiotic resistance can influence natural selection").* Although this experiment does not explicitly demonstrate antibiotic resistance, it gives students a visual representation of how antibiotics should work in the presence of bacteria and leaves room for higher-order thinking to deduce reasoning for potential problems when antibiotics do not work (i.e. due to resistance). (North Carolina Department of Public Instruction, 2024, p. 4)

Upon teacher discretion, the phage portion of this lesson can be omitted and/or implemented separately from the environmental swab portion, as the swab experiment aims to simply visualize asexual reproduction.

Sources

North Carolina Department of Public Instruction. (2024, May). *Biology: 2023 support document* (K–12 Science Support Documents). https://www.dpi.nc.gov/documents/cte/curriculum/science/biology-standards/open

Dr Jeffers-Francis Lab materials

Appendices

See attachments

- *E. coli* Phage Isolation Student Lab Sheet
- *E. coli* Phage Isolation Reflection Sheet
    - Modified lab/reflection sheet
- Microbial Case Study Scenarios
- Bacteria vs Phage Presentation Rubric