

A Simple Exercise for Teaching Bacteriophage Concepts in the Undergraduate Laboratory Using Commercially Available Disinfectant †

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INTRODUCTION

Bacteriophages are viruses that infect specific bacterial species, hijacking the infected bacterium's machinery to multiply and eventually burst its prey, resulting in zones of clearing on the culture plate known as plaques (1). Viruses are difficult to visualize due to their small size, visible only with electron microscopes (2). Many teaching facilities are unable to access this specialized equipment, and other techniques must therefore be employed to explore viruses in undergraduate teaching laboratories. One of the most common detection methods used is a plaque assay, whereby bacteria and bacteriophages are mixed on an agar plate and the plaques are observed to assess whether lytic viruses specific for the bacterial hosts are present (2). The plaque assay is a key laboratory method in virology for the isolation and enumeration of phage particles (3), as well as for measuring virulence of the bacteriophages, and studying the growth of lytic viruses (4). The assay can also be used for the purposes of phage typing, whereby phages are used to identify pathogenic bacteria in diagnostic laboratories. Finally, there is renewed interest in the therapeutic use of phages to treat antibiotic-resistant pathogenic bacterial infections (5). Teaching students about plaque assays provides an understanding of the concepts of viral plaque formation and the host specificity of bacteriophages, comprising an important intellectual pillar in teaching microbiology.

We have designed a simple laboratory exercise in a single practical class for a second-year biomedical science course to demonstrate the plaque assay and to use phage typing to differentiate three bacterial strains of *Staphylococcus aureus*. This phage typing exercise was designed as

a demonstration due to the large class size and also due to the number of other microbiology techniques taught within the same practical class. To mimic viral plaques, we used a commercially available laboratory disinfectant, TRIGENE (diluted at a 1:100 ratio), which is capable of clearing bacterial growth on the agar plate, resulting in a visual effect similar to that of lytic phages on bacterial lawns. The teaching strategy involved analyzing phage typing of three different culture plates (with different configurations of artificial plaques, Fig. 1) of *Staphylococcus aureus*, which students were told had been isolated from three hypothetical hospital wards.

This low-cost exercise effectively facilitated student–student and student–instructor interactions, engaging students to link theory to practical exercises in order to interpret phage typing results and to discuss the answer to the question provided in the students' laboratory manual. This created an effective collaborative learning situation in the classroom for teaching students about bacteriophages without the use of real phages.

This exercise is suitable for students with undergraduate experience in microbiology, virology, or associated laboratory techniques whose prior lectures have covered introductory aspects of microbiology, viruses, and bacteriophages along with safety measures taught during the course and during the laboratory exercise.

PROCEDURE

Materials and methods

Groups of four students were provided with three culture plates labeled “ward #1,” “ward #3,” and “ward #6,” showing phage typing (Fig. 1) of *S. aureus* strains isolated from three different hospital wards. Preparation of culture plates showing phage typing activity and detailed instructor notes are available in Appendix I. Student instructions were provided in each student's laboratory manual (Appendix 3). Clearly labeled biohazard bags were available for waste disposal, and disinfectant was available to disinfect benches.

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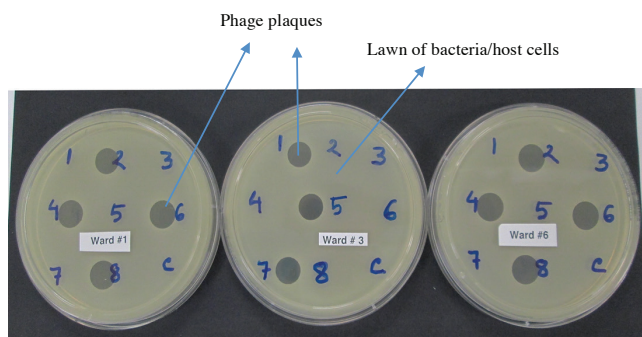


FIGURE 1. Student materials per group. Culture plates with simulated bacteriophage plaques showing phage typing of hypothetical *Staphylococcus aureus* strains isolated from three different wards: wards #1, #3, and #6 in the case study hospital.

Measuring students' learning

This exercise was scheduled to be completed within a 15-minute period of a two-hour microbiology laboratory with a class size of 120 students. Students were instructed to record phage typing results of all *S. aureus* strains/wards as outlined in Table 1 (Appendix 1). Although many plaques are generally round, they can be variety of shapes and sizes (1). A plaque is indicated by a zone of no growth (clear plaque) in the lawn of *S. aureus*. If phages 1, 5, and 7 gave a positive result (clear plaques), the phage type would be recorded as 1/5/7.

The results were discussed by groups of students, focusing on answering the question outlined in Table 2 (Appendix 1), aiming for engagement of students in active learning about the relationship between bacteria and viruses, as well as to encourage teamwork and the development of critical thinking skills. Students were also given the opportunity to question the teaching assistants if required and to learn how to both convey and receive constructive criticism/feedback from their peers as well as their instructors.

Safety issues

Students had already received adequate training about laboratory safety and been introduced to all basic microbiology laboratory techniques such as aseptic technique, culture transfer, staining, bacterial isolation and purification, making a lawn plate, biochemical identification, and safe handling of BSL1 and BSL2 microorganisms, etc. during their previous microbiology laboratory sessions and lectures before performing this practical exercise (please see prerequisite student knowledge, Appendix 1). The bacterial strain (*Staphylococcus aureus* subsp. *aureus* ROSENBAACH ATCC 35556) used for this laboratory exercise is classified as a BSL2 organism, and students therefore handled all bacterial lawn plates according to ASM biosafety BSL2 level guidelines (https://www.asm.org/images/asm_biosafety_guidelines-FINAL.pdf). Students performed this activity with appropriate personal protective equipment (i.e., lab coat, closed-toed

shoes, gloves, and safety glasses). After completing this practical exercise, students disinfected lab-bench surfaces, all biohazard wastes were disposed of according to the university biohazard waste disposal guideline, and finally, students thoroughly washed their hands in compliance with ASM guidelines.

CONCLUSION

This phage typing exercise is easy to prepare and can easily be adapted by any institution as it is or with modification (Appendix 2) since it does not require expensive equipment, and the materials that are needed to prepare culture plates to show the phage-typing activity are inexpensive and can easily be sourced from scientific suppliers. TRIGENE disinfectant can be used as an effective tool to mimic bacteriophage plaques, reliably teaching students about phage diversity, bacteria–virus interaction, and the process of viral plaque formation in an easily reproducible manner. This quick and easy trick saves time as well as eliminating the need to source bacteriophages specific for different bacterial strains.

SUPPLEMENTAL MATERIALS

- Appendix 1: Instructor's notes
- Appendix 2: Possible modification
- Appendix 3: Student's laboratory protocol

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