An Undergraduate Laboratory Activity Demonstrating Bacteriophage Specificity

Mary E. Allen¹ and Ruth A. Gyure²*
¹Department of Biology, Hartwick College, Oneonta, NY 13820, ²Department of Biological and Environmental Sciences, Western Connecticut State University, Danbury CT 06810

Bacteriophage are among the most diverse and numerous microbes inhabiting our planet. Yet many laboratory activities fail to engage students in meaningful exploration of their diversity, unique characteristics, and abundance. In this curriculum activity students use a standard plaque assay to enumerate bacteriophage particles from a natural sample and use the scientific method to address questions about host specificity and diversity. A raw primary sewage sample is enriched for bacteriophage using hosts in the family Enterobacteriaceae. Students hypothesize about host specificity and use quantitative data (serial dilution and plaque assay) to test their hypotheses. Combined class data also help them answer questions about phage diversity. The exercise was field tested with a class of 47 students using pre- and posttests. For all learning outcomes posttest scores were higher than pretest scores at or below p = 0.01. Average individualized learning gain (G) was also calculated for each learning outcome. Students' use of scientific language in reference to bacteriophage and host interaction significantly improved (p = 0.002; G = 0.50). Improved means of expression helped students construct better hypotheses on phage host specificity (G = 0.31, p = 0.01) and to explain the plaque assay method (G = 0.33, p = 0.002). At the end of the exercise students also demonstrated improved knowledge and understanding of phage specificity as related to phage therapy in humans (p < 0.001; G = 51).

INTRODUCTION

Viruses are an important topic in any microbiology class and also one of the more difficult for students to visualize given their size. Most undergraduate laboratories lack access to an electron microscope so instructors must rely upon indirect methods for visualizing viruses. One of the most common of these is the plaque assay, in which host cells mixed with virus particles are immobilized in a gel matrix (e.g. agar). When infected cells lyse, viral particles infect neighboring cells and eventually an area clear of cells, referred to as a plaque, becomes visible. First described as a method for isolating bacteriophage (1, 4, 5) the assay was later modified (2) for use with animal viruses. Today the standardized plaque assay is commonly used in microbiology teaching laboratories (7).

Bacteriophage present an ideal system for development of curricular exercises that use the plaque assay because their host cells are easier and safer to grow in the laboratory than animal cells. In this laboratory activity students use a plaque assay to detect the presence of bacteriophage in a natural environmental sample—human sewage effluent. Sewage is a perfect environment to search for phage with hosts in the intestinal tract, and these include genera such as Escherichia, Enterobacter, Serratia, and Alcaligenes. In preparation for the exercise, different bacterial species and strains are mixed with primary sewage effluent to enrich for phage, which are collected in a phage suspension by filtering out the bacteria. Students are asked to hypothesize the outcome of mixing a phage suspension with a bacterial host. Hypotheses should differ depending upon whether students are provided with a bacterial host that is the same species or strain used to enrich for phage in the suspension, prompting students to consider host specificity of bacteriophage.

Although most phage in the prepared suspensions are specific to the host microbe added to the phage enrichment culture, diverse phage from the original sewage are also present in low numbers. Furthermore, phage in the environmental sample display varying host ranges. For example some may infect multiple genera in a family, and others only a single species. This adds a degree of variation to the results that is common in studies of natural systems and therefore more realistic than what is generally presented by “cookbook” laboratory exercises. Students are consequently led to explore questions about host specificity and host range of bacteriophage, as well as viral diversity, and to apply their knowledge to other systems including phage therapy.

©2013 Author(s). Published by the American Society for Microbiology. This is an Open Access article distributed under the terms of the a Creative Commons Attribution – Noncommercial – Share Alike 3.0 Unported License (http://creativecommons.org/licenses/by-nc-sa/3.0/), which permits unrestricted non-commercial use and distribution, provided the original work is properly cited.
Intended audience

This laboratory activity has been used in separate one-semester undergraduate microbiology courses designed for preallied health students and for upper-level biology students. The activity would also be appropriate for courses addressing reproduction and cultivation of viruses, viral host-cell specificity and applications of serial dilutions to experimental studies.

Learning time

Two laboratory periods, each three hours long, are used to complete this activity. During the first laboratory period students hypothesize the outcomes of mixing a specific bacterial host with a phage suspension (enriched with a bacterium identical to or different from their host microbe). They also prepare serial dilutions of phage and use them for a plaque assay. In the second laboratory period students examine plates for visible plaques, calculate phage concentrations in the original phage suspension, and complete a worksheet with questions that address the learning objectives of the activity. At least 24 hours between laboratory sessions is necessary for phage to reproduce and form visible plaques.

Prerequisite student knowledge

Ideally, before starting this activity, students should have basic knowledge of the scientific method, aseptic technique, serial dilution procedures, and safe practices for working at BSL2 (3). Before the second laboratory period students should be familiar with the plate-count method for enumerating microorganisms, dilution factor calculations, and phage structure, reproduction, and host-cell specificity. The activity has also been successfully utilized in an introductory 200-level lab, wherein this activity was used to teach these principles for the first time. In this case, instructors should be prepared to spend more time on explanations and demonstrations during the lab periods.

Learning objectives

After successful completion of this exercise, students will be able to:

1. Recognize and identify characteristics of bacteriophage that distinguish them from other viruses and from bacteria.
2. Construct hypotheses, then generate and analyze data related to questions about phage/host specificity.
3. Use appropriate scientific language when referring to phage and their hosts.
4. Apply knowledge and understanding of phage specificity to questions related to phage therapy in humans.
5. Evaluate how phage are cultivated and quantified.
6. Demonstrate appreciation for and understanding of phage diversity.

PROCEDURE

Materials and equipment for one class of 20 students working in pairs:

- Approximately 300 ml primary sewage sample (incoming)\(^{a}\) collected in a sterile sealable container. Each enrichment starts with 30–50 ml sewage and, after being filtered, results in approximately 10–20 ml lysate (phage suspension). Each student pair will require at least 3 ml of their assigned lysate. Note, a phage lysate is a liquid solution with a high concentration of phage particles usually obtained by repeated rounds of phage infection and cell lysis. In this case the lysate is obtained through overnight incubation with enrichment using appropriate host cells.
- 5–10 disposable 50-ml plastic tubes or urine collection cups, one for each phage enrichment desired.
- 5–10 disposable filters with bases, 0.45-μm pore size. One filter unit for each phage enrichment, and extras because during filtration these easily get clogged. Source of vacuum for the filtration is also necessary. For filter units without bases separate sterile glass sidearm flasks may be used to collect the lysate.
- 40–60 disposable and sterile 15-ml plastic tubes, for dispensing of host cultures and phage lysates to students. Also needed for mixing top agar with host/phage mixture before plating (3 per student pair) and these must have screw caps.
- 100 ml tryptic soy broth for setting up enrichments.
- 40 or more tryptic soy agar plates, 3 per pair of students plus extras for growing starting cultures.
- 20–30 tubes of sterile dilution fluid, simple 0.85% sodium chloride (non-iodized) works well, 2 tubes of 9 ml each for each student pair plus extras. Glass tubes are sufficient here but if they do not have screw caps use caution when preparing the dilution series and do not allow vortexing (mix each with a pipette).
- 40 small tubes each with 4 ml of 0.5% soft top agar, 3 per pair of students plus extras. To prepare 100 ml, mix 0.5 g agar with 3.0 g tryptic soy broth powder, boil, dispensing into tubes, and autoclave. Keep molten until first laboratory meeting or re-autoclave to liquify and cool to 50°C before needed.

\(^{a}\)Primary incoming sewage is untreated and can be obtained from a local sewage treatment plant upon request. The treatment plant operator/director is typically quite cooperative and should of course be contacted beforehand to obtain permission and to explain the educational purpose for collecting the sample.
• 40 disposable sterile 1-ml pipettes (at least 3 per student pair) and pipetting devices.
• 60 sterile 3-ml transfer pipettes, at least 5 per student pair.
• Water bath to keep top agar at 50ºC during the first laboratory period, shaking water bath for sewage enrichments, standard 37ºC incubator for plates.
• Bunsen burners for flaming tubes, if desired.
• BSL2 laboratory capability (includes use of gloves, goggles and biowaste containers).

Student instructions

Prior to arriving for the first laboratory meeting students are assigned to read the Student Laboratory Protocol: Bacteriophage from a Natural Environmental Sample (Appendix 1) and instructed to bring this handout with them to the laboratory session. Appendix 2 (Student Worksheet: Bacteriophage from a Natural Environmental Sample) is handed out to students at the start of the laboratory period, at which time they fill out the sections under the heading “Information from Period 1.” If students keep laboratory notebooks they might be asked to record the same information there.

Faculty instructions

This exercise is designed for a lab of 20 students working together in pairs and can be easily scaled up or down. Each student pair is provided with one species or strain of host bacteria and a phage lysate (obtained by filtration of an overnight enrichment with a particular bacterial host). Various student pairs are given different host and phage combinations. For example, *Escherichia coli* might be the bacterial host given to two groups who then each receive phage suspensions derived from different enrichment cultures. One group would have phage lysate from an enrichment culture seeded with *Escherichia coli* strain B, and the other would have phage from an enrichment culture seeded with *Serratia marcescens*. During the activity it is important that students and instructors do not confuse the specific bacteria provided as the host with the ones used to generate the phage lysate. Clearly printing CULTURE or HOST and LYSATE or PHAGE on the relevant tubes, and bacterial names in parentheses, is important. Using different types of tubes, color coding, etc. is also helpful.

The number of different host/phage combinations used in the exercise depends upon the number of different bacteria used in phage enrichment cultures, which are the same bacteria provided to students as bacterial hosts. For example, the use of five bacterial genera or strains will result in 25 different host/phage combinations. Bacteria that have proven reliably successful in meeting the learning goals for this activity are *Escherichia coli*, *Escherichia coli* strain B, *Escherichia coli* strain C, *Serratia marcescens* and *Enterobacter aerogenes*. Other bacteria commonly found in human sewage can be used in addition or as substitutes but safety issues specific to these different bacteria should be taken into account. Only a few possible pairings are given in Table 1 and the likelihood of producing plaques is shown in the 4th column. A good idea is to include several examples of pairings that produce plaques, along with some that do not, in order to meet the goals of this learning activity. If there are

### TABLE 1.
Potential pairings.

| Student Pair | Culture<sup>a</sup> <br>(host used in student activity) | Lysate<sup>b</sup> <br>(host used for enrichment) | Likelihood of Producing Visible Plaques Using the Recommended Dilutions |
|--------------|------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| 1            | *Escherichia coli*                             | *Escherichia coli*                              | High                                            |
| 2            | *Serratia*                                     | *Escherichia coli*                              | Low                                             |
| 3            | *Escherichia coli* strain B                    | *Escherichia coli* strain B                     | High                                            |
| 4            | *Escherichia coli* strain B                    | *Escherichia coli*                              | Low                                             |
| 5            | *Escherichia coli* strain C                    | *Escherichia coli* strain C                     | High                                            |
| 6            | *Enterobacter*                                 | *Enterobacter*                                  | High                                            |
| 7            | *Escherichia coli*                             | *Serratia*                                      | High                                            |
| 8            | *Serratia*                                     | *Enterobacter*                                  | Low                                             |
| 9            | *Escherichia coli*                             | *Enterobacter*                                  | Low                                             |
| 10           | *Serratia*                                     | *Serratia*                                      | High                                            |

<sup>a</sup>Potential host bacteria used on the day of the activity, freshly grown as an overnight culture. <sup>b</sup>Possible matched lysate with the name of the species or strain used in the phage enrichment step.
not enough student pairs in the class, each may be assigned more than one host/lysate pairing, or the instructor may set up additional host/lysate pairings for class comparison.

**Detailed preparation instructions**

**Up to one week before the activity.** Collect sewage sample and store at refrigeration temperature (see Fig. 1).

**Three days before the first laboratory period.** Streak each bacterial species or strain onto a trypticase soy agar plate and incubate at 37°C for ~24 hours.

**Two days before the first laboratory period.** Set up phage enrichment cultures by adding 20–30 ml of primary sewage to sterile cups or flasks with headspace for aeration. Add 3 ml sterile trypticase soy broth and inoculate the mixture from one of the overnight bacterial plate cultures. Separate enrichment cultures should be set up for each bacterial species or strain, and all incubated overnight at 37°C with shaking for aeration.

**One day before the first laboratory period.**

a) Collect phage lysate from each enrichment culture by vacuum filtration through a sterile disposable filter with an attached base (see Fig. 2). As an alternative to bases, separate sterile glass sidearm flasks may be used. Filters with 0.45-μm pores are shown to be sufficient to accomplish the goals of this activity, and 0.2-μm filters are not practical due to clogging. Three ml of each phage suspension is then dispensed into sterile tubes to be handed out to student pairs. The filtering step can be time consuming and 15–20 minutes may be necessary to collect 10 ml of lysate from one culture. Typically instructors can expect to recover a maximum of 15 ml of phage solution from each enrichment culture.

b) Also prepare liquid overnight cultures of the bacterial host cultures so they will be fresh on the morning of the activity. Bacteria can be grown overnight in 5 ml of broth in separate tubes for each student pair, or as a single, larger volume culture. The latter will require instructors to dispense cultures into sterile tubes before the start of the laboratory period. Log-phase cultures work best for this lab but even early stationary phase cultures will result in some plaques. In the case of multiple lab sessions some culture can be kept growing into log phase in a shaking incubator throughout the day and distributed to students as needed. Do not forget to clearly label these tubes as CULTURE or HOST and to label the viral lysates as LYSATE or PHAGE (see Fig. 3).

**The day of the first laboratory period.** Each student pair is provided with a tube of phage lysate and a tube of fresh bacterial host culture as per the assigned pairing. Also at each station: three tubes each containing 9 ml saline; three empty 15-ml tubes with screw caps; three disposable sterile 1-ml pipettes and pipetting device; five or six disposable sterile transfer pipettes; three trypticase soy agar plates; gloves, goggles, and biohazard container (see Fig. 4). In nearby water bath, provide beakers with three tubes of top agar each, held at 50°C until needed. At time of use...
students will remove a beaker and the water it contains will keep the tubes warm while working at their bench. Note that in some labs glass tubes and flasks are used. However, disposable screw cap tubes are safer and more effective for mixing. This is especially important for mixing top agar with host bacteria.

The day of the second laboratory period. At the second laboratory meeting student pairs retrieve their plaque assay plates and continue following instructions from Appendix 1, Student Laboratory Protocol. They will observe plaques and determine numbers of plaque forming units in their original phage solutions and use this information to complete the sections of the Student Worksheet (Appendix 2) under the heading “Questions for Period 2 and Results.” So that students can quickly and easily identify plaques, instructors may want to assign them to access the web resource “Plaque Assay: Assorted Views of Bacteriophage Plates” (6) before they arrive to start the laboratory. Students should be reminded that plaques are not always perfectly round or clear, and there is some variation in size and appearance (i.e. some plaques are cloudy, some have a clear center with cloudy halo, etc.).

The last questions on the Student Worksheet (Appendix 2) address results compiled from all student pairs. If there are 10 or more student pairs the class dataset will be large enough for students to draw sound conclusions and students can be instructed to record their results on a blackboard or other surface visible to the class. Data from different laboratory sections may also be combined and handed out to students to use in answering questions in the days following the laboratory meeting. As students consider how to interpret class results (which will vary depending on the particular phage present in the sewage and the host bacteria used), they may need to consider topics such as host specificity and host range of bacteriophage, and diversity of phage in sewage. Examples of data that would require such consideration are provided in Table 2, and discussed in “Sample Data,” below.

Additional information for instructor

Students should arrive at the first laboratory period having read the Student Laboratory Protocol (Appendix 1). They will also need this handout with them in the laboratory. At the start of the laboratory period the instructor hands each student pair tubes of phage lysate and host bacterial species or strain, and the Student Worksheet (Appendix 2). Students begin by completing the sections of the worksheet under the heading “Information from Period 1,” which asks them to hypothesize the outcome of mixing their phage and bacterial host. As stated previously, it is important that students do not confuse the specific bacteria provided as the host with the one used for enrichment of the phage, so checking with each student pair to ensure their understanding is recommended. Once student pairs have recorded complete hypotheses and appropriate justifications they can begin the plaque assay method, following instructions provided in Appendix 1, Student Laboratory Protocol. For a detailed explanation of the plaque assay method, and some very good sample photos, instructors can refer to Panec and Katz (7).

In this exercise students are instructed to plate phage from the original suspension and at final dilutions of $10^1$, $10^2$, and $10^3$. In our experience this results in at least one plate with 30–300 plaque-forming units (if the phage is specific
to the host) most of the time. Sometimes phage concentrations are high enough that the highest dilution plate has too many plaques to count, so if time and materials are available, plating an additional dilution of $10^{-4}$ may be desirable. At the end of the first laboratory period plaque assay plates should be incubated at 37ºC until visible plaques form, after which they can be kept at 4ºC until the second laboratory period.

Suggestions for determining student learning

Student learning may be assessed based upon completion and quality of answers given on the Student Worksheet (Appendix 2). An instructor’s key is provided with the worksheet in Appendix 2. Students are given this handout during the first laboratory meeting and, in our classes, return it one week after the second laboratory meeting. Student learning may also be addressed by pretesting, including the same material as posttest questions on any examination covering the material. An example of a pre-/posttest designed to match learning goals for use in the field-testing is included as Appendix 3 (Pre- and Posttest: An Undergraduate Laboratory Activity Demonstrating Bacteriophage Specificity), along with an instructor’s key.

Sample data

Results of plaque assays completed by students in microbiology at Hartwick College are provided in Table 2. More plaques formed when the host bacterium was the same as the one used to enrich the phage lysate, which was the result most students expected. Students were also asked to look for, and explain, additional patterns in the data. This encouraged them to think about additional aspects of phage biology and ecology.

For example, students noticed that phage in the lysate enriched with Enterobacter aerogenes appeared to have the narrowest host range. This lysate resulted in fewer plaques, when mixed with bacteria other than E. aerogenes, than the other phage lysates produced when they were mixed with hosts different from the bacteria used for their enrichment. Students might also be encouraged to notice that when comparing all pairings where the host bacterium was the same as that used to enrich the phage lysate, the E. aerogenes-enriched phage lysate contained the smallest number of plaque forming units. This suggests the E. aerogenes-enriched phage reproduce more slowly than phage in the other enrichments or it may reflect a smaller starting population size in the sewage.

Data in Table 2 illustrate that the results students collect in this exercise will depend upon the specific host-lysate pairings handed out by instructors and, to a smaller extent, on geographic variation in sewage.

Safety issues

This experiment must be carried out in a laboratory equipped for handling microorganisms at BSL2. The instructor is referred to Biosafety Guidelines for Handling Microorganisms in the Teaching Laboratory: Development and Rationale (3) for further details. The primary sewage is handled only by the instructor. Resulting lysates used for preparing the dilution series are safe for students to handle using recommended BSL2 practices.

DISCUSSION

Field testing and evidence of student learning

For approximately three semesters, this curriculum exercise has been used in separate one-semester undergraduate microbiology courses designed for preallied health students and for upper-level biology students at Hartwick College and Western Connecticut State University (WCSU). The activity was field-tested in fall 2012 at WCSU in a class of biology and preallied health students. A pretest (Appendix 3) was administered before the start of the first laboratory period and the same questions were included as a posttest on a class examination the week after the second laboratory meeting. Both pre- and posttest results were available for 47 students and these were used in analyses of learning gain. A rubric (Appendix 4) was developed to measure, on a scale of 1–4, the learning gain for each learning outcome (LO) based upon answers to questions on the pre- and posttests. The second learning outcome addresses three
aspects of experimental design—hypothesis development, methodology, and results prediction—and gain was assessed separately for each of these.

For all learning outcomes the average rubric scores for the posttest were higher than for the pretest (Fig. 5). Two-tailed t-tests indicated all these differences were significant at, or below, \( p = 0.01 \). Normalized learning gains (\( G = (\text{posttest score} - \text{pretest score})/(4 - \text{pretest rubric score}) \)) were also calculated for each student from the rubric scores. Averages for each learning outcome are provided in Table 3.

One of the highest normalized gains was in LO3 (\( G = 0.51 \)), use of appropriate scientific language when referring to phage and their hosts. Improved means of expression helped students construct better hypotheses on phage host specificity (LO2a: \( p = 0.01; G = 0.31 \)) and to explain the plaque assay method (LO2b: \( p = 0.002; G = 0.33 \)). For example, the percentage of students who correctly described the results of a phage assay (Question 5 on pre- and posttests, Appendix 3) as plaque forming units/ml increased from 24% on the pretest to 85% on the posttest. Examples of pretest and posttest hypotheses proposed by students are provided in Table 4. Students were also better at predicting the results of a plaque assay after the exercise (LO2c: \( p < 0.001; G = 0.28 \)) and had an improved understanding of phage cultivation and quantification (LO5: \( p < 0.001; G = 0.24 \)).

Students’ ability to apply their knowledge of the plaque assay to interpret data improved but remained their greatest weakness. When asked to use an image of a plaque assay dilution plate to determine the number of phage in the original sample, on the posttest only 16% of all students answered correctly although this was significantly higher than the 0% correct answers on the pretest. Encouraging students in a class to record results for plaque assay plates of other groups would provide more practice in this skill and might improve their overall learning gain.

### Possible modifications

Students often have difficulty understanding the concept of serial dilution. Many references are available to help prepare students for this activity, but a suggested worksheet is included here (Appendix 5). It is designed to match the protocol used in this activity and help the students understand how to calculate the concentration of phage in their own starting lysates. An instructor’s key is also provided.

This activity works best (and has been field-tested) using sewage obtained from a wastewater treatment plant as described. However, there are alternative sources that could be considered. Locations identified as point sources for possible coliform contamination exist in areas where treatment plant overflow occurs, especially along the coasts of lakes, streams and other waterways where plant outlets discharge after a heavy rain. This water is likely to

![FIGURE 5. Rubric scores for six targeted learning outcomes. Averages (n = 47) of student scores on a rubric (Appendix 4) used to measure knowledge of the six learning outcomes on a scale of 1–4. Learning was measured with pre- and posttests. Learning outcome 2 is divided into three parts: LOH = hypothesis, LOM = method, LOA = analysis. Two-tailed t-tests indicated differences between all pre- and posttest averages were significant at, or below, \( p = 0.01 \).](image)

| Learning Outcomes | Normalized Learning Gains |
|-------------------|---------------------------|
| 1. Recognize and identify characteristics of bacteriophage that distinguish them from other viruses and from bacteria. | 0.38 |
| 2a. Construct a hypothesis related to phage/host specificity. | 0.31 |
| 2b. State methodology for testing a hypothesis related to phage/host specificity. | 0.33 |
| 2c. Predict result to support hypothesis constructed about phage/host specificity. | 0.28 |
| 3. Use appropriate scientific language when referring to phage and their hosts. | 0.50 |
| 4. Apply knowledge and understanding of phage specificity to questions related to phage therapy in humans. | 0.51 |
| 5. Evaluate how phage are cultivated and quantified. | 0.24 |
| 6. Demonstrate appreciation for and understanding of phage diversity. | 0.33 |

\( ^* \) Assessment of learning outcome 2 was divided into three parts (a–c).

\( ^b \) Averaged normalized individual student learning gains (G) for each learning outcome. \( G = (\text{posttest rubric score} - \text{pretest rubric score})/(4 - \text{pretest rubric score}) \). A rubric (Appendix 4) was used to measure change in learning knowledge between pre- and posttests, on a scale of 1–4.
contain coliforms and bacteriophage. Be sure that anyone collecting or handling this water wears gloves and takes proper precautions. Another possibility could be fresh fecal matter from a healthy, vaccinated, indoor domestic pet such as a cat. This material should be highly diluted (about 0.5 g per 100 ml media) before beginning the enrichment process. No matter where the source material comes from, BSL2 protocol must be utilized at all times.

If one wishes to extend this activity into a multi-week project, phage from the resulting plates can easily be isolated. These “cleaner” individual phage lysates can then be used to test, more definitively, their ability to infect a variety of additional strains, species, and genera of hosts.

Isolation of phage from plaques:

1. Prepare small microcentrifuge tubes, each containing 100 μl sterile phage buffer (autoclave a solution of 10 ml 1 M Tris (pH 7.5), 4 g NaCl, 10 ml 1 M MgSO₄, 980 ml water).
2. Using a sterile micropipette tip, carefully and gently scrape the center of one well-isolated plaque. It is fine if some agar gets on the tip but try not to touch the region beyond the edge of the plaque. Transfer this material to one of the prepared microcentrifuge tubes by dispensing material in tip and mixing tip vigorously in the buffer.
3. If desired, centrifuge these tubes at 10,000 rpm for 1 minute and transfer supernatant to a clean sterile tube. The small pellet will consist of bacterial cells that may have come along with the plaque.
4. Use 10, 25, or 50-μl aliquots of this isolated lysate as the inoculum (in place of the 0.1 ml diluted sewage lysate) according to the protocol already described in this activity.

Any well-isolated plaque can be used, and plaques with different morphologies are ideal. These new lysates can be used to inoculate a variety of additional bacterial lawns in student-designed experiments to better test the specificity hypotheses.

SUPPLEMENTAL MATERIALS

Appendix 1: Student laboratory protocol: bacteriophage from a natural environmental sample
Appendix 2: Student worksheet: bacteriophage from a natural environmental sample (includes instructor’s key)
Appendix 3: Pre- and posttest: an undergraduate laboratory activity demonstrating bacteriophage specificity (includes instructor’s key)
Appendix 4: Grading rubric for pre- and posttest
Appendix 5: Student supplementary worksheet: understanding serial dilution

ACKNOWLEDGMENTS

The authors declare that there are no conflicts of interest.
REFERENCES

1. d’Herelle, F. 1917. Sur un microbe invisible antagoniste des bacilles dysenterique. Acad. Sci. Paris 165:373–375.
2. Dulbecco, R., and M. Vogt. 1953. Some problems of animal virology as studied by the plaque technique. Cold Spring Harb. Symp. Quant. Biol. 18:273–279.
3. Emmert, E. A. B., and the ASM Task Committee on Laboratory Biosafety. 2013. Biosafety guidelines for handling microorganisms in the teaching laboratory: development and rationale. J. Microbiol. Biol. Educ. 14:78-83.
4. Gratia, A. 1936. Numerical relations between lysogenic bacteria and particles of bacteriophage. Ann. Inst. Pasteur 57:652.
5. Hershey, A. D., G. Kalmanson, and J. Bronfenbrenner. 1943. Quantitative methods in the study of the phage-antiphage reaction. J. Immunol. 46:267–279.
6. Katz, D. S., and M. Panec. 2006. Plaque assay: assorted views of bacteriophage plates. American Society for Microbiology. http://www.microbelibrary.org. Accessed 22 September 2012.
7. Panec, M., and D. S. Katz. 2006. Plaque assay protocols. American Society for Microbiology. http://www.microbelibrary.org. Accessed 21 September 2012.