Cornell Institute for Biology Teachers
in partnership with the
Pseudomonas-Plant Interaction Project

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Title: **PPI Module 2: Bacterial Conjugation**

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Appropriate Level: High School: Life Science, Honors, or Advanced Placement Biology

Abstract: This classroom activity demonstrates interactions between two different strains of bacteria. Students will design an experiment that demonstrates that genetic information can be passed from one species of bacteria to another.

Time Required: This experiment is broken up into three parts, each of which can be fit into a 45-minute period. The first activity involves plating three different types of bacteria on two plates containing antibiotic medium. These plates are then incubated for 48 hours and analyzed on the second session. The bacteria are then mixed and plated again. They are analyzed around 48 hours later in the third part of the experiment.

Special Needs: Bacterial cultures and plates of antibiotic media (see Materials and Methods)

NYS Learning Standards **1- Inquiry, Analysis and Design:** 2- Testing proposed explanations: 2.3a,b; 3- Analyzing data: 3.3; 3.4a; **4- Living Environment**: 2- Inheritance: 2.1d,h; 3- Evolution: 3.1b,h; 4- Reproduction: 4.1b

Additional Teacher Information

Copies of the lab and other information can be found in the High School Connect section of the PPI website, at: http://pseudomonas-syringae.org.

Materials and Methods

It is recommended that students work in groups of two or three.

Materials:

- Marker (one per student group)
- Sterile Water (at least 2 ml per student group)
- Microfuge tubes (four per student group)
- Pipette droppers (four per student group)
- Bacterial Cultures- *P. syringae, E. coli*, and helper strain
- Disposable loops
- Media plate with no antibiotics (one per student group)
- Media plate with antibiotic A (one per student group)
- Media plate with antibiotic B (one per student group)
- Media plate with antibiotics A and B (one per student group)

Pre-Lab Discussion Suggestions:

- Different methods of genetic transfer in bacteria- conjugation, transformation, transduction
- Why antibiotic resistance arises in hospitals
- Selective pressure
- Overuse/Abuse of Antibiotics
- Formulating Good Hypotheses

Answers

Analysis Questions:

1. A **hypothesis** is essentially an educated idea or guess on what you think or expect the answer to a question to be. Your will either will or will not be supported by the results of your experiment. What is your in the above experiment?

   In this case the hypothesis will most likely either be that P. syringae is resistant to Antibiotic A and E. coli is resistant to Antibiotic B or that they aren't (or possibly that one or both of the bacteria are resistant to both of the antibiotics).

2. What information did you base your on?

   The previous work sited at the beginning of the lab.

3. What results do you expect based on your ?

   Their results should support the hypothesis.

4. What other results might you see and how would they change your ?

   Responses to this question will vary just as long as the results support the alterations to the hypothesis.

Post-Lab Questions:

1. Based on your observations from session 1, which bacteria grew on the plate with both anitbiotics that you plated on session 2? How do you account for this growth?

   The only bacteria that should grow on this plate with both antibiotics are the bacteria that were allowed to conjugate. The way this experiment is set up the P. syringae should have picked up an antibiotic resistance gene from the E. coli through conjugation.

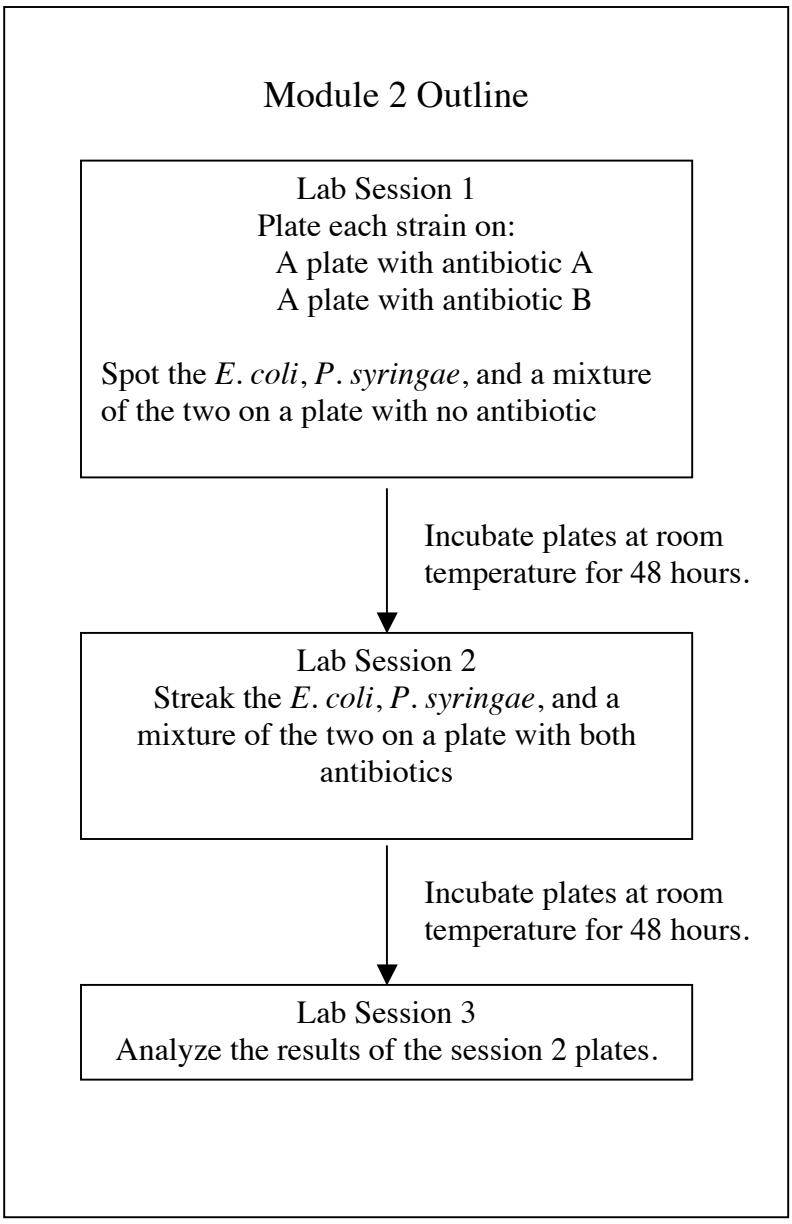
2. There are two ways a bacteria can be resistant to an antibiotic: they can either develop a spontaneous mutation or have an enzyme which breaks down the antibiotic. Spontaneous mutations occur at random and are reletively rare, while genes for anitbiotic resistance enzymes are often able to be transferred from one bacteria to another. Which one of these did the bacteria in this module have?

   The bacteria in this experiment transferred the gene for an enzyme that breaks down the antibiotic. If the antibiotic resistance was the result of a spontaneous mutation, we would expect to see colonies appear where the unmixed bacteria were plated.

3. What problems can the over use of antibiotics cause? Explain how these problems are caused.

   If bacteria are challenged with an antibiotic and one cell in the population acquires resistance, it will flourish while the other cells are killed off. Resistance can easily develop in bacteria, which can then transfer the resistance to other (possibly pathogenic) bacteria. This is the reason it is necessary for patients and doctors to exercise caution about when to use antibiotics. Antibiotics are

a precious resource in our fight against bacterial pathogens and it is important that future generations be able to use them too.



PPI: Module 2
New York State Learning Standards

Standard 1: Inquiry Analysis and Design

Key Idea 2: Beyond the use of reasoning and consensus, scientific inquiry involves the testing of proposed explanations involving the use of conventional techniques and procedures and usually requiring considerable ingenuity.

2.3 – Develop and present proposals, including formal hypotheses to test explanations; i.e., predict what should be observed under specific conditions if the explanation is true.

a. Hypothesis are predictions based upon both research and observation

b. Hypotheses used to determine what data to collect and as a guide for interpreting the data.

Key Idea 3: the observations made while testing proposed explanations, when analyzed using conventional and invented methods, provide new insight into natural phenomena.

3.3 – Assess the correspondence between the predicted result contained in the hypothesis and actual result, and reach a conclusion as to whether the explanation on which the prediction was based is supported.

3.4 – Based on the results of the test and through public discussion, revise the explanation and contemplate additional research.

a. Hypotheses are valuable, even if they turn out not to be true

Standard 4: Living Environment

Key Idea 2: Organisms inherit genetic information in a variety of ways that result in continuity of structure and function between parents and offspring.

2.1 – Explain how the structure an replication of genetic material result in offspring that resemble their parents.

d. Asexually produced offspring are normally genetically identical to the parent.

h. Genes are segments of DNA molecules. Usually and altered gene will be passed on to every cell that develops from it.

Key Idea 3: Individual organisms and species change over time

3.1 – Explain the mechanisms and patterns of evolution

b. New inheritable characteristics can result from new combinations of existing genes

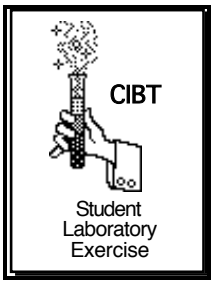
h. The variation of organisms within a species increased the likelihood that at least some members of the species will survive under changed environmental conditions.

Key Idea 4: The continuity of life is sustained through reproduction and development.

4.1 – Explain how organisms including humans reproduce their own kind.

b. Some organisms reproduce asexually with all the genetic information coming from one parent.

PPI Module 2:
Transfer of Genetic Information Between Bacteria



Experimental Objective

It is known that bacteria can easily acquire resistance to new antibiotics. In this experiment we will test if antibiotic resistance can be transferred between bacteria that are put in close proximity to each other.

Methods

Lab Session 1

Previous studies of bacteria have shown that that *P. syringae* is resistant to Antibiotic A and *E. coli* is resistant to antibiotic B. Today we will test if these bacteria can transfer antibiotic resistance to each other through the process of conjugation. First, we will confirm their antibiotic resistance by streaking each of these bacteria on plates containing antibiotics A or B. This experiment also includes a helper strain, which increases the efficiency of the conjugation process, but is not resistant to any antibiotics.

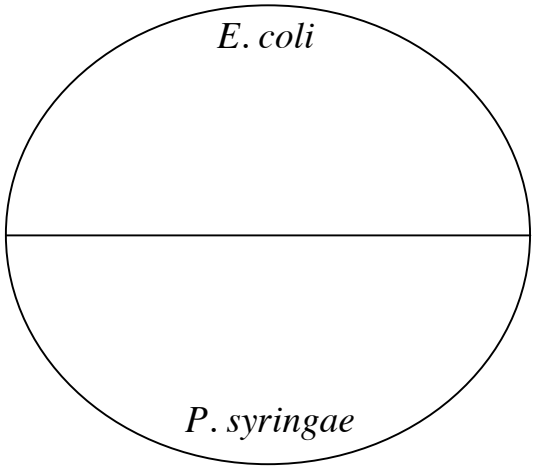
Test to Confirm Antibiotic Resistance

1. You will need to get three bacterial plates, one with antibiotic A, one with antibiotic B, and one with no antibiotic. Your instructor will also give you access to two plates with bacteria growing on them. One plate will have a culture of *P. syringae* and the other will have a culture of *E. coli*. Take some time to observe the bacteria and write some of the distinguishing features of each culture in the space below.

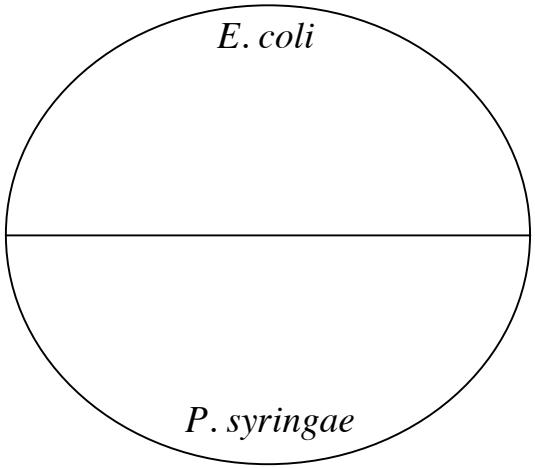
   - *E. coli*:

   - *P. syringae:*

2. Label one Antibiotic A plate and one Antibiotic B plate as shown below:

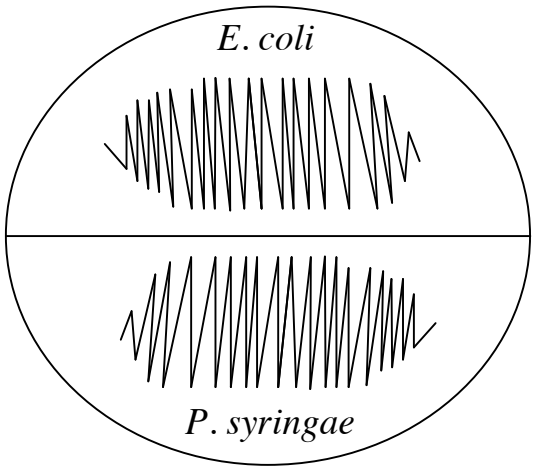


Media + antibiotic A

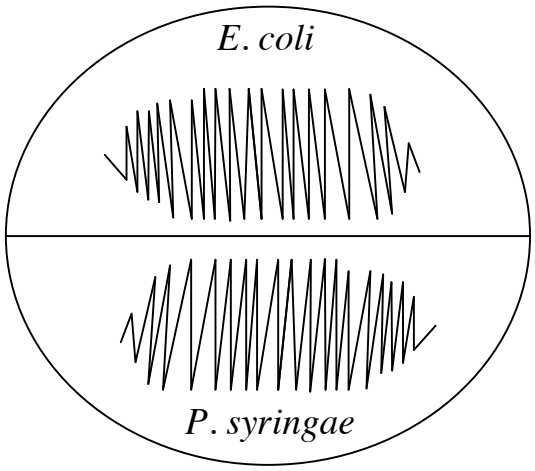


Media + antibiotic B

3. Using a disposable loop, streak *P. syringae* or *E. coli* on the plates in the pattern shown below:



Media + antibiotic A



Media + antibiotic B

Session 1 Analysis

1. An **hypothesis** is essentially an educated idea or guess on what you think or expect the answer to a question to be. Your hypothesis will either will or will not be supported by the results of your experiment. What is your hypothesis in the above experiment?

2. What information did you base your hypothesis on?

3. What results do you expect based on your hypothesis?

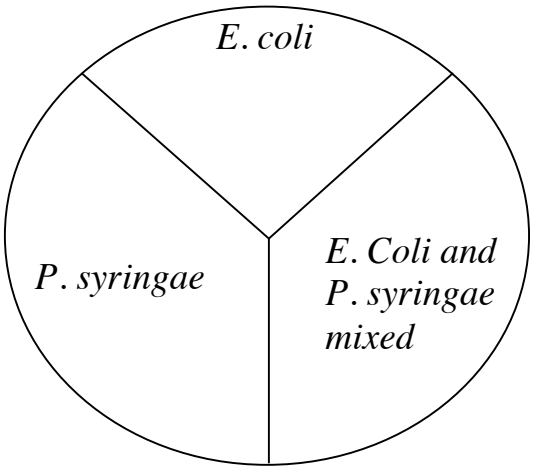
4. What other results might you see and how would they change your hypothesis?

Setting up the Conjugation

Next we will test to see if antibiotic resistance can be transferred from one strain to another.

1. Label three microfuge tubes: *P. syringae*, *E. coli*, or Mix.

2. Label the bottom of a media plate (no antibiotics) as shown below:



Media (no antibiotic)

3. Using a transfer pipette, fill each tube with approximately 500 µl sterile water (the tubes are graduated).

4. Using a loop, scrape a generous amount of cells off of the *P. syringae* plate and shake off in the *P. syringae* micro-centrifuge tube.

5. Dispose of the *P. syringae* loop as instructed to by your instructor.

6. Take a clean sterile loop and repeat this for the *E. coli*.

7. Using a pipette dropper, pipette the water in the tubes up and down to unclump the cells. Before disposing of the pipette dropper, put of drop of each suspension (*P. syringae* or *E. coli*) into the C tube and then a drop onto the media plate where you labeled. Try to combine approximately equal amounts of each of the three bacteria in the Mix tube (remember to use a different dropper for each different type of bacteria).

8. Gently shake the Mix tube to mix.

9. Using a new pipette dropper, spot a drop of the cell suspension from the conjugation mixture onto the media plate where you labeled Mix.

10. Let dry and keep at room temperature for 48 hours.

Lab Session 2

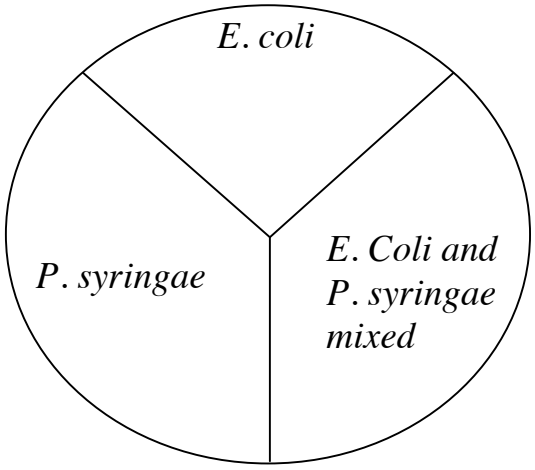
1. Before you begin, check the plates you streaked during session 1. What do you see? Which bacteria were able to grow on:

    a. Media with antibiotic A:

    b. Media with antibiotic B:

    c. Media with no antibiotic:

2. Label one media plate with antibiotics A and B with *P. syringae*, *E. coli*, and Mix as shown below:



Media with antibiotics A and B

3. Streak the bacteria spot from the plate from session 1 (with no antibiotics) in the corresponding space on the plate with both antibiotics.

4. Incubate your plate at room temperature for at least 48 hours and answer the following questions.

    a. What is your hypothesis for the above experiment?

    b. What information did you base your hypothesis on?

    c. What results do you expect based on your hypothesis?

    d. What other results might you see and how would they change your hypothesis?

Lab Session 3

Check the plate with both antibiotics for growth. Did you see growth of:

- *E. coli*
- *P. Syringae*
- The mixture

Post-Lab Questions

1. Based on your observations from session 1, which bacteria is growing on the plate with both antibiotics that you plated on session 2? How do you account for this growth?

2. There are two ways a bacteria can be resistant to an antibiotic: they can either develop a spontaneous mutation or have an enzyme which breaks down the antibiotic. Spontaneous mutations occur at random and are reletively rare, while genes for anitbiotic resistance enzymes are often able to be transferred from one bacteria to another. Which one of these did the bacteria in this module have?

3. What problems can overuse of antibiotics cause? Explain how these problems are caused.