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

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Title: **PPI Module 1: Plant-Bacterial Interactions**

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

Abstract: This classroom activity demonstrates interactions between plants and specific strains of *Pseudomonas* (a plant-pathogenic bacteria). Students will design an experiment that demonstrates the specificity of the hypersensitive response. This serves as a starting point to learn the importance of model systems through comparisons of two pathogens. Both *Pseudomonas*, a plant pathogen, and *Yersina pestis*, the bacteria that causes the bubonic plague, use the same molecular machinery to infect cells.

Time Required: Two 45-minute periods separated by one to two days. During the first period have your students set up the experiment and inoculate the plant leaves. During the second period they observe the development of the hypersensitive response (HR) and analyze their results.

Special Needs: Special items needed (see below).

NYS Learning Standards **1-Inquiry, Analysis, and Design:** 2- Testing proposed explanations: 2.1; **4-Living Environment**: 5-Dynamic Equilibrium: 5.2a-c,j5.3a,b; 6-Interdependence: 6.1g. **6-Interconnectedness, Common Themes**: 2- Models: 2.2

Additional Teacher Information

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

Materials and Methods

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

Materials:

- Leaf/Leaves *
- Beaker (1 per group)
- Bacterial culture plates (*P. syringae* pv. *tomato* and *P. fluorescens*)
- Graduated Test Tubes (3 per group. 15 ml conical tubes are good for these.)
- Water (~50 ml per group)
- Disposable culture loops (~8 per group)
- Marker
- Dissecting needle (1 per group)
- Plastic pipette droppers
- Turbidity Standard or Spectrophotometer

* This can be one tobacco leaf or a cutting with three chrysanthemum leaves

Module 1, Section A

Post-Lab Questions

1. What control is used in this experiment and why?

   The water sample is one control in this experiment. It shows that there is nothing in the water or the infiltration process that causes the plant to exhibit HR.

2. What is the hypersensitive response (HR)?

   The hypersensitive response is suicide, or apoptosis, of the infected cells in order to stop the pathogen from spreading by cutting off the its food supply.

3. Why does P. syringae pv. tomato cause disease on tomato but HR on tobacco?

The resistance proteins in the tomato plant are unable to recognize the effector proteins that P. syringae pv. tomato *injects into plants. Tomato plants are unable to defend themselves from* P. syringae pv. tomato *through the HR. Tobacco, on the other hand, has resistance proteins that are able to recognize the effector proteins that* P. syringae pv. tomato *and defend itself through the HR.*

4. Do you think you would see HR in nature?

   In nature, bacteria are present in small numbers, then a few cells in the leaf commit suicide. The death of of a few cells is very small and there is no change in the leaf that is visible to the naked eye.

5. Why is *P. syringae* a good model system? Give an example of another model system.

   P. syringae is a good model system because the method it uses to infect plants that is common to many plant and animal pathogens. It is also useful because it is not pathogenic or dangerous for people to work with, unlike Y. pestis. It is also easily cultured and grows well in a lab.

6. What bacterium caused the black plague? How is the hypersensitive response and the black plague connected in a way that makes scientists studying each work closely together?

   Yersinian pestis is the bacterium that causes the black plague. Both Y. pestis and P. syringae use the type III secretion system to attack their hosts cells. Studying how P. syringae functions can give us insight into how Y. pestis can infect people.

Module I, Section B

Post-Lab Questions

1. What concentrations of *P. syringae* did you test to see if they exhibited a visible HR?

   The answer to this question will depend on what concentrations the students tested. A series of 1:2 dilutions will usually give a good result when starting with a concentration of OD600 of 1.

2. What factors did you have to take into account when you determined which concentrations of bacteria to test?

   It is important to pick concentrations that are far enough apart that you will see some difference between the concentrations you test, but close enough together that the data is useful (ie. In this specific example, the samples tested gives you a good idea of the concentration where the transition between a visable and invisible HR occurs).

3. What concentrations *P. syringae* did you find exhibited a visible HR?

   The answer to this question will depend on what concentrations the students tested.

4. What controls did you use in your experiment, and what information did you get from them?

   The answer to this question will depend on what concentrations the students tested.

5. How did you estimate the areas of your HR lesions? What factors could introduce error in your area calculation?

Assumptions made about the shape of the HR lesion (or the weight of a section of leaf if the students chose that method) will introduce error into calculations.

6. Assume that a leaf cell is a cube that is 30 µM in all dimensions, and that a leaf is 5 leaf cells thick. How many leaf cells were in the HR patch of the lowest concentration of bacteria that exhibited a HR?

   The formula used to calculate this would be:
   ((Area of HR lesion) x (150 µM)) / (27000 μM^3) = (number of leaf cells in the HR lesion)

7. How many bacterial cells did you infiltrate into that leaf? Since it is difficult to measure the exact volume you infiltrated, due to leaking, you can estimate the amount of bacteria infiltrated based on the size of the HR lesion. Assume that the area of the infiltrated bacterial solution matches that of the HR lesion, and was 50 µM thick. Also assume that a bacterial solution with an OD600 of 1 has a concentration of 1,500,000 bacteria/ml.

   The formula used to calculate this would be:
   (Area of HR lesion) x (50 µM) = (volume of bacterial solution)
   (volume of bacterial solution) x (concentration of bacterial solution) = (number of bacterial cells infiltrated into a leaf)

8. From the numbers you just calculated, how many bacteria per plant cell does it take to cause a visible HR to occur?

   The formula used to calculate this would be:
   (number of bacterial cells infiltrated into a leaf) / (number of leaf cells in the HR lesion) = (number of bacterial cells per leaf cell)

PPI Module 1
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.

2.1- Devise ways of making observations to test proposed explanations.

Standard 4: Living Environment

Key Idea 5: Organisms maintain a dynamic equilibrium that sustains life.

5.2- Explain disease as a failure of homeostasis

a. Homeostasis is constantly threatened. Failure to respond can result in death

b. Infection of plants by Viruses, bacteria and fungi

c. Immune response to antigens

j. Research generates knowledge to diagnose, prevent, treat, control or cure diseases

5.3- Relate processes at the system level to the cellular level in order to explain dynamic equilibrium in multicelled organisms.

a. Dynamic equilibrium results from detection and response to stimuli

b. Feedback mechanisms have evolved that maintain homeostasis

Key Idea 6: Plants and animals depend on each other and their physical environment

6.1- Explain factors that limit growth of individuals and populations

g. Relationships between organisms may be negative, neutral, or positive.

Standard 6: Interconnectedness and Common Themes

Key Idea 2: Models are simplified representations of objects, structures or systems used in analysis, explanation, interpretation, or design.

2.2- Collect information about the behavior of a system and use modeling tools to represent the operation of the system.

PPI Module 1: Plant-Bacterial Interactions

CIBT
Student Laboratory Exercise

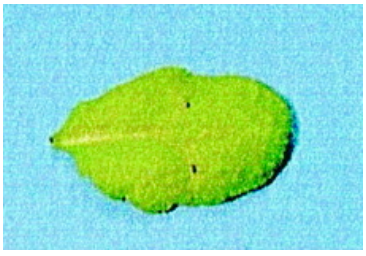
Module 1, Section A

Experimental Objective

Inoculate leaves with *Pseudomonas syringae* pv. *tomato* and *Pseudomonas fluorescens* and evaluate the different responses.

Methods

1. Cut off one large leaf or several small leaves from the plant provided. Place your cutting in water in a beaker or cup.
2. Label three graduated test tubes: *P. syringae*, *P. fluorescens*, or water.
3. Add 10 ml of water to each test tube.
4. Using a disposable loop, scrape some bacteria from the plate labeled *P. fluorescens*. Shake off the cells in the tube labeled *P. fluorescens*. *Remember to dispose of loop in autoclave bucket after each use!
5. Vortex tube to un-clump cells. If you don't have access to a vortex, pipette gently up and down with the plastic transfer pipette to break up the cell clump.
6. Adjust the density by adding more cells or water until you reach an OD600 = 1 . OD stands for Optical Density, and is a measure of how much light of a particular wavelength (in this case 600 nM) a solution blocks. You can measure OD using a spectrophotometer or by comparing the bacterial suspension to a turbidity standard.
7. Repeat steps 4-6 for *P. syringae*.
8. Choose six sections on the leaf (choose areas between veins). Label each section with a marker: W for water, Pf for *P. fluorescens,* or Ps for *P. syringae* (2 replicates for each).
9. With a dissecting needle, poke very small holes into the center of each section on the bottom of the leaf. (Make sure to not make the hole in a vein.)



10. Suck up some *P. fluorescens* bacterial suspension with the plastic pipette dropper. Put your finger underneath the hole you just made for support. Place the dropper on top of the hole and apply some pressure but not enough to break through the leaf. Squeeze the pipette. You should see liquid spreading throughout the inside of the leaf.
11. Repeat Step 10 for both *P. syringae* and water.
12. Leave cuttings on the bench top and examine after 48 hours.

Results

Record your observations. You may use words or drawings:

P. fluorescens	*P. syringae*	Water

Post Lab Questions

1. What control is used in this experiment and why?

2. What is the hypersensitive response (HR)?

3. Why does *P. syringae* pv. *tomato* cause disease on tomato but HR on tobacco?

4. Do you think you would see HR in nature?

5. Why is *P. syringae* a good model system? Give an example of another model system.

6. What bacterium caused the black plague? How is the hypersensitive response and the black plague connected in a way that makes scientists studying each work closely together?

Module 1, Section B

Experimental Objective

Determine the concentration of *Pseudomonas syringae* pv. *tomato* necessary to trigger a visible hypersensitive response in the test plant.

Materials and Methods

Work in groups of 3.

Materials

- Leaves*
- 1 Beaker
- Bacterial culture plates (*P. syringae* pv. *tomato* and *P. fluorescens*)
- Graduated Test tubes (~10-15 ml) (~6-8/group)
- Water
- Disposable culture loops
- Marker
- Dissecting needle
- Plastic pipette droppers
- Turbidity Standard

* You will have to determine the number of leaves your group will need based on your experimental design. If you are using a plant with large leaves, such as tobacco, you can perform multiple infiltrations on one leaf. You will need more leaves (or entire stems) if you are using plants with smaller leaves, such as mums.

Experimental Design

The Hypersensitive Response (HR) that plants exhibit is a specific reaction to effector molecules injected by pathogenic bacteria. If the bacteria are present in small numbers, then a few cells in the leaf commit suicide. In this case, there is no change in the leaf that is visible to the naked eye. On the other hand, if an excessive number of bacteria are infiltrated into a leaf a large number of cells will commit suicide. It is possible that so many plant cells commit suicide that there are not enough cells remaining to maintain healthy tissues. In this case, all the cells in the infiltrated area die and a large brown-grey splotch appears on the leaf.

In this lab we are going to set up and conduct an experiment to determine how many bacteria it takes to cause the plant to exhibit a visible HR. In order to do this we will need to test different concentrations of bacterial solution to see if they cause a visible HR response.

Experimental Procedures

It is very important to plan out an experiment before actually starting it. Not only do you need to know how much materials you will need, but also need to make sure that you will be able to understand your results. For this experiment, you will attempt to determine the concentration of bacteria needed to exhibit a visible HR. You will find a discussions of techniques that you may find useful for this experiment under "Experimental Techniques: Making Dilutions and Estimating Area" in the PPI Supplemental Reading Packet.

Experimental Setup

Discuss with your group how you want to setup your experiment before you begin. Record your experimental design below.

Dilutions of *P. syringae* to test:

Controls to test:

Total number of test/samples:

Number of leaves needed:

Results

In the space below, design a chart that accurately displays the samples that you tested and the results of your experiment. *The chart should include the following information: Bacteria type, concentration, HR (Y/N), area of leaf affected*.

Post-Lab Questions

1. What concentrations of *P. syringae* did you test to see if they exhibited a visible HR?

2. What factors did you have to take into account when you determined which concentrations of bacteria to test?

3. What concentrations *P. syringae* did you find exhibited a visible HR?

4. What controls did you use in your experiment, and what information did you get from them?

5. How did you estimate the areas of your HR lesions? What factors could introduce error in your area calculation?

6. Assuming that a leaf cell is a cube that is 30 μM in all dimensions, and that a leaf is 5 leaf cells thick. How many leaf cells were in the HR patch of the lowest concentration of bacteria that exhibited a HR?

7. How many bacterial cells did you infiltrate into that leaf? Since it is difficult to measure the exact volume you infiltrated, due to leaking, you can estimate the amount of bacteria infiltrated based on the size of the HR lesion. Assume that the area of the infiltrated bacterial solution matches that of the HR lesion, and was 50 μM thick. Also assume that a bacterial solution with an OD600 of 1 has a concentration of 1500,000 bacteria/ml.

8. From the numbers you just calculated, how many bacteria per plant cell does it take to cause a visible HR to occur?