“Veggie Soup” Food Spoilage by Microbes

Science in the Real World Microbes In Action

“Veggie Soup” is a curriculum unit developed as part of the Science In The Real World: Microbes In Action Program. The curriculum units were developed with support from the National Science Foundation, The Coordinating Board of Higher Education, Sigma Chemical Company, Pfizer Foundation and the Foundation for Microbiology.

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At A Glance

Description:

The scientific method is used in this laboratory exercise to demonstrate that a bacterium called *Erwinia carotovora* is the organism that causes a disease called “soft rot”. This laboratory is a model for the study of infectious disease; however, this organism is harmless to humans.

Time Requirements:

This exercise requires two 40 – 45 minute laboratory periods.

Curriculum Placement:

This unit can be used to teach scientific method, an introduction to infectious disease, or as an introduction to microorganisms.

Materials:

(for class of 30 working in pairs)

- 15 diseased carrot slices
- 10% bleach solution
- 45 petri dishes (disposable sterile polystyrene)
- 15 permanent-marking pens
- Paper towels
- 15 pairing knives
- Water – tap and distilled
- 15 600 ml. beakers
- 15 scissors
- 15 forceps
- 15 toothpicks

A selection of the following fruits or vegetables:

turnip	snow pea	asparagus
yam	ginger root	radish
apple	celery	onion
squash	cucumber	potato
carrot	green pepper	

Veggie Soup!!!!!!

Background:

Vegetables are an important source of nutrients for all of us. They always play an important role in a good balanced diet. We eat them for snacks and with meals. But sometimes when you reach into your refrigerator for a piece of fruit or a vegetable you pull out one that may have rotted. How and why did it go bad? Often these are caused by a bacteria called *Erwinia carotovora,* which is the organism that causes a disease called "soft rot". Soft-rotting bacteria are common in the environment and as a result you may find it in your vegetable bin.

Under warm, moist conditions the disease spreads rapidly, the optimal temperature for bacterial multiplication being approximately 25°C. This bacteria will also grow in the refrigerator but much more slowly . The bacteria are commonly found on or in the tubers of plants and become active when conditions occur that are favorable for disease development.

Question:

Could *Erwinia carotovora* infect all of the fruits and vegetables in the vegetable bin?

Materials:

(for one lab group)

1 "diseased" slice of carrot
3 petri dishes
paper towels
1 pairing knife
water – tap and distilled
600 mL beaker
10% bleach solution
1 permanent-marking pen
scissors
forceps
toothpick

2 different fruits or vegetables – choose from the following:

turnip	snow pea	asparagus
yam	ginger root	radish
apple	celery	onion
squash	cucumber	potato
carrot	green pepper	

Procedure

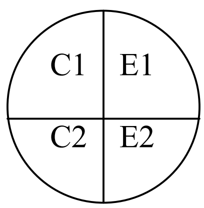
Day 1

1. Obtain 1 sterile petri dish. Using the permanent marker, write all of the lab partners initials in a small area on the bottom of the petri dish.

2. DO NOT OPEN THE PETRI DISH. Place the petri dish on top of paper towels stacked 3 layers thick. Using a pencil, trace the bottom of the petri dish onto the paper towels in three different places.

3. Cut out the three sets of traced circles.

4. Using a pencil, label the paper towel as shown below.



C1= Control fruit or veggie #1
C2= Control fruit or veggie #2
E1= Experimental infected fruit or veggie #1
E2= Experimental infected fruit or veggie #2

5. Place the stack of three cut out paper towels in the bottom of the petri dish.

6. Moisten the paper towels with water.

7. Choose 2 different fruits or vegetables that you would like to test and obtain them from your teacher.

8. Using the permanent marker write the name of each of the different fruits or vegetables in a small area on the bottom of each of the petri dishes.

9. Fill a 600 mL beaker with a 10 % bleach solution.

10. Dip a clean paper towel into the bleach solution and wipe off the counter where you will be working.

11. Wash one of the <u>whole</u> fruits or vegetables in the 10% bleach solution and immediately rinse with water. Dip your knife in the bleach and rinse with water.

12. Carefully cut 2 slices of one of the fruits or vegetables (approximately 5 to 8 mm thick and 5 to 8 mm square). Make sure the slices are small enough that they would not touch each other or the lid of the petri dish.

13. Dip the forceps in the bleach and rinse with water.

14. Use the forceps to pick up each of the pieces of the fruit or vegetable. Carefully place the slices in their proper place in the petri dish.

15. Observe the culture of *Erwinia carotovora.* Remove the lid of the petri dish. Using a toothpick, gently scrape a small but visible amount of bacteria and gently spread it over the surface of your fruit or veggie labeled E1. Discard the toothpick in the container provided by your teacher.

16. Repeat step 15 for your fruit or vegetable labeled E2.

17. **Do not** infect C1 or C2

18. Place the petri dishes in the area designated by your teacher.

20. Wash the table top with the bleach water. Put all equipment back in its proper places and clean up the lab area. Wash your hands.

<u>Day 2</u>

1. Obtain your petri dishes from the area where they were stored. Observe the samples that you infected with the diseased carrot. Compare the slices that were exposed to the bacteria with the control slice.

2. Use a toothpick to touch the food slices if rotting is not visible.

3. Record your observations in Table 1. Also, record your observations of your classmates
food items.

4. Dispose of your materials as directed by your teacher.

Name______________________________

Date ______________________

Data Table

Name of Fruit or Vegetable	Observations

Results and Analysis

1. Did the “control carrot” (C) show any signs of disease? Explain what you see.

2. Do you think soft rot is an infectious disease? Explain your answer.

3. Why was it necessary to wash the knife and the outside of each of the fruits and vegetables with the 10% bleach solution?

4. Why did you put a control in each of your dishes?

5. If you discover soft rot in your vegetable drawer, what precautions could you take to prevent it from infecting the other vegetables in your drawer? How could you prevent further rot from occurring in that drawer?

6. Pool data from the entire class. Were all of the different fruits and vegetables infected by the bacteria? Give a possible reason for what you see.

7. Based on this experiment, state a question/problem that you would like to solve about fruits and vegetables. Design an experiment that would help you find the answer.

Teacher Background

As long ago as the 16^{th} century, it was thought that something unseen could cause disease. Even in ancient times, it was recognized that disease could be contagious (individuals with leprosy were segregated from health people). Ignaz Semmelweis and Joseph Lister recognized the importance of bacteria in causing disease, but added little data in support of the "germ theory". In 1876 Robert Koch was studying a disease of cattle called anthrax, and found convincing evidence that the disease was caused by a bacterium that was named *Bacillus anthracis*. In studying this disease he established criteria and a method for determining whether a microorganism actually caused a disease. This method is called Koch's Postulates. By 1883 he had isolated many microorganisms from air, water and soil and found that they had little significance in disease. In fact, of the tens of thousands of different microorganisms that inhabit every ecological niche on the earth, only a few hundred or so can cause disease. In addition to establishing criteria for disease Koch also established techniques that we still use today for the isolation of bacteria in pure culture.

Microorganisms that cause disease are called pathogens. Diseases that are readily transferred from an infected individual to a non-infected individual are called communicable diseases. One route of transmission of disease is through contact, either directly with an infected individual, with aerosols produced by coughs or sneezes, or sometimes with a contaminated object, such as a handkerchief used by someone with a disease. Another mechanism of disease transfer is via a vector such as an insect that may bite first an infected individual and then an uninfected individual. Malaria is transmitted by mosquitoes. Some rare diseases are transmitted directly through animal bites (e.g., rabies virus). Many diseases are transmitted through food and water and some through air. Only a few diseases are both deadly and easily transmitted. Good hygiene and a healthy body protect most people from diseases caused by microorganisms.

Our understanding of microorganisms and how to grow and identify them was significantly advanced by the work of Robert Koch and by Louis Pasteur. The development of sewage and water treatment facilities removed a major source of pathogenic organisms in daily life. Good sterile techniques and improved hygiene in hospitals decreased deaths from childbirth and surgical procedures. The identification and manufacture of antibiotics and the development of vaccines have significantly reduced communicable diseases in the 20^{th} century. Significantly, however, communicable diseases are still a major problem in third world countries where sanitation is poor and drugs are not readily available.

Instructional Objectives:

At the end of this unit of activities the student should be able to:

1. demonstrate the methods of scientific inquiry by
   a. stating a problem
   b. writing a hypothesis
   c. performing an experiment
   d. gathering and organizing data
   e. analyzing data
   f. developing further investigations

2. demonstrate the following laboratory skills
   a. use of sterile techniques
   b. comparing and contrasting
   c. transferring bacteria

3. demonstrate the following concepts
   a. an understanding of Koch's postulate by describing the transmission of a bacterial disease

Sources of Supplies

Carolina
2700 York Road
Burlington, NC 27215
(800) 334-5551

Description	Stock number	Quantity	Cost
Petri dishes	F6-70-3033	500	$95.00
Culture of *Erwinia carotovora*	F6-15-5045	1 tube	$ 7.00

Preparations:

- At least a week before the lab, obtain a culture *of Erwinia carotovora.* Store culture capped in the refrigerator.
- Two days before the lab, streak the bacteria onto sufficient nutrient agar plates so that you have one for every two groups.
- Prepare 10% bleach solution in advance. (Warn the students that bleach can discolor their clothes.)

Teacher Hints and Trouble shooting

1. This organism does not require an incubator. It will grow at room temperature (20 – 22°C) but the results may take longer. If you have an incubator, 25°C is an ideal growth temperature and will give the best results.

2. If visible results are not seen within 24 hours, allow additional time for the incubation. Results appear to depend on the concentration of the *Erwinia* culture.

3. Infected food samples can be thrown into the trash.

4. The following are sample results that the students may observe:

   NO EFFECT - Snow pea, ginger root, apple, onion

   MEDIUM INFECTION – Asparagus and radish

   STRONG INFECTION – Turnip, yam, celery, squash, cucumber, potato, green pepper, and carrot

Teacher Answer Guide

1. Did the "control carrot" (C) show any signs of disease? Explain what you see.

   The control carrot should show no signs of disease. If they exist, ask the students to look for areas where the sample may have touched other samples.

2. Do you think soft rot is an infectious disease? Explain your answer.

   Yes, the disease was spread from the original carrot to many other samples.

3. Why was it necessary to wash the knife and the outside of each of the fruits and vegetables with the 10% bleach solution?

   To remove any bacteria that may have already contaminated them.

4. Why did you put a control in each of your dishes?

   To determine whether the fruit or vegetable would rot or decay even though it was not inoculated.

5. If a bacterium is called *Erwinia carotovora* , what do you think the primary target would be?

   Perhaps Carrots (perhaps from the name Carrot-ovora)

6. Pool data from the entire class. Were all of the different fruits and vegetables infected by the bacteria? Give a possible reason for what you see.

   Answers will vary but hopefully not all are infected. Reasons for not being infected could include:
   - natural resistance
   - waxy cuticle that prevents infection
   - high sugar content that may inhibit growth

7. Based on this experiment, state a question/problem that you would like to solve about fruits and vegetables. Design an experiment that would help you find the answer.

   Answers will vary. Look for a controlled experiment.

Extended Activities

Have students investigate how different kinds of bacterial soaps and disinfectants destroy bacteria. Students could test those fruits or vegetable that became infected with the disease. They could prepare solutions of different kinds of bleaches, soaps, hand cleaner, etc. and dip the fruit or vegetable in it (like they did with the 10% bleach solution) and then infect it with the bacteria and observe whether soft rot occurs or not.

Science Connections:

Ecology –
The ecosystem, both on land and in the water, depends heavily upon the activity of bacteria. The cycling of nutrients such as carbon, nitrogen, and sulfur is completed by their ceaseless labor.

Organic carbon, in the form of dead and rotting organisms, would quickly deplete the carbon dioxide in the atmosphere if not for the activity of decomposers. Decomposition is the breakdown of these organisms, and the release of nutrients back into the environment, and is one of the most important roles of the bacteria.

The cycling of nitrogen is another important activity of bacteria. The primary way in which nitrogen becomes available to plants is through nitrogen fixation by bacteria. Other denitrifying bacteria metabolize in the reverse direction, turning nitrates into nitrogen gas or nitrous oxide. When colonies of these bacteria occur on croplands, they may deplete the soil nutrients, and make it difficult for crops to grow.

Cross-Curriculum Ideas

MATH – Calculations of percent of fruits or vegetable tested became infected with the disease.

ENGLISH- Write the conclusion to the laboratory in English class and check proper grammar and punctuation.

SOCIAL STUDIES- Investigate bacterial epidemics that may have occurred in history.