Bacterial Soft Rot of Vegetables

Learning Outcomes

Students will: (1) learn about enzymes in bacteria that break down structural molecules leading to soft rot in tissues, (2) learn about and use inoculation and isolation methods associated with bacterial plant pathogens, (3) demonstrate the effect of environmental factors (temperature) on soft rot development, and (4) demonstrate the "rules of proof of pathogenicity" (Koch's postulates).

QCC Standards

Grade 7:

Science Process Skills Standards 1 & 2

15.1 Lists harmful and beneficial effects of the organism based on similarities of characteristics.

17.1 Describes and compares various life processes of plants, e.g., ferns and seed plants (gymnosperms vs. angiosperms).

Applied Biology & Chemistry 1:

37.1 Describes three roles that microorganisms can take in nature and in industry.

37.2 Cultures microorganisms that you sample from food, air, water, and soil on prepared media.

37.3 Identifies six types of relationships that microorganisms can have with one another and with other organisms.

37.4 Gives examples of species relationships in which microorganisms participate.

37.11 Investigates the cause and prevention of a plant disease.

Applied Biology & Chemistry 2:

Science Process Skills Standards 1 & 3

12.1 Locates the main vegetative and reproductive parts of plants.

12.3 Matches the abnormal appearance of plant leaves, stems, or roots to the disease, pest or nutritional condition that is causing the change in appearance.

18.6 Identifies the factors in harvesting that can affect crop yield and quality.

Biology:

Science Process Skills Standards 1 & 3

6.4 Describes the four basic types of organic compounds (carbohydrates, lipids, proteins, and nucleic acids) and their functions in the cell.

15.1 Describes the cellular structure and the conditions necessary for growth and reproduction (of monerans).

19.1 Lists and describes distinguishing characteristics of gymnosperms and angiosperms.

19.4 Describes the importance of seed plants for food, medicine, and other products.

25.1 Identifies and explains the interactions of biotic and abiotic factors in an ecosystem.

Environmental Science:

Science Process Skills Standards 1 & 3

25.1 Recalls the biotic and abiotic factor in the ecosystem.

28.1 Identifies the factors that affect the balance of nature.

29.3 Evaluates the role of microorganisms in agriculture, such as soil formation, fertility, and recycling carbon, oxygen, phosphorus, iron, nitrogen, and sulfur.

Microbiology:

Science Process Skills Standards 1 & 3

18.1 Analyzes food spoilage in terms of causes, processing, and storage consideration.

18.3 Investigates methods of food preservation.

19.4 Analyzes the harmful effects of microbes in agriculture.

National Standards

The atoms and molecules on the earth cycle among the living and nonliving components of the biosphere.

Energy flows through ecosystems in one direction, from photosynthetic organisms to herbivores to carnivores and decomposers.

Organisms both cooperate and compete in ecosystems. The interrelationships and interdependencies of these organisms may generate ecosystems that are stable for hundreds or thousands of years.

Background & Definitions

Soft rot of fleshy vegetables and ornamental plants is caused by the bacterium Erwinia carotovora var. The bacterium is a Gram-negative rod, approximately 0.7x1.5um and has peritrichous flagella. It is non-spore forming and facultatively anaerobic. It grows well in nutrient agar and nutrient broth but not above 36 degrees C. The soft rot bacteria can grow and are active over a range of temperatures from 3-35 degrees C but are killed by extended exposure at about 50 degrees C.

Soft rot occurs worldwide wherever fleshy storage tissues of vegetables and ornamentals are found. Potatoes, carrots, and onions are among the most affected vegetables along with tomato and cucumber. Iris bulbs (and similar ornamentals) are often affected. The disease can be found on crops in the field, in transit, and in storage or during marketing resulting in great economic losses. Soft rot causes greater total loss of produce than any other bacterial disease.

The soft rot bacterium enters plant tissues primarily through wounds, often created by insect feeding or bruising at harvest. Insects and water are effective in spreading the bacterium. Once in the plant tissue, the bacterium produces increasing amounts of pectolytic enzymes that break down the pectic substances of the middle lamella (which cements cells together), causing break down and maceration of the tissues. Slimy masses of the bacteria and accompanying cellular debris Òoozes Ó out from cracks in the tissue and becomes visible on the surface. Tissue in the affected area becomes Òmushy,Ó cream colored, and slimy. Water diffuses into the intercellular spaces and cells plasmolyze and die. The bacteria continue to move and multiply in the intercellular spaces and cells plasmolyze and die. The bacteria continue to move and multiply in the intercellular spaces and produce more enzymes for further tissue invasion and maceration. The end result is that infected tissues become soft and a slimy mass, often with a ÒputridÓ odor. An entire potato tuber or other vegetable may become converted into this watery, decayed mass within 3-5 days! Because of the dramatic effects on host tissue and the rapidity of disease development, soft rot is an excellent model to illustrate plant disease development to students.

Rules of Proof of Pathogenicity

The rules of proof of pathogenicity were formulated in 1882 by the German scientist Robert Koch (hence, KochÕs postulates) from his studies on animal anthrax. These rules were adapted to also determine the actual cause of plant disease. Briefly states, the rules are as follows:

1. Observe whether there is a constant association between the Òsuspected pathogenÓ and disease symptoms.

2. Isolate the suspected pathogen in pure culture and identify it.
3. Inoculate the organism into healthy plant tissue (some species, variety, etc.) and observe whether the symptoms produced are the same as those of the original disease (Step 1).
4. Re-isolate the pathogen from the inoculated plant tissue (step 3) in pure culture and compare it with the original isolated culture (step 2).

This process can require a considerable amount of time (several weeks to months) but this experiment permits completion in approximately two weeks.

Materials & Equipment
Two white potatoes, carrots, and onions (per 3-4 students)
Filter paper disks (sterile, if possible)
Knife
Soft-rot bacterium (Erwinia carotovora var) culture (available through commercial supply companies)
Nutrient agar plates
Saran wrap/ Parafilm
Indelible pen
Teasing needles
Transfer loops
Incubation facilities (refrigerator, 26 degrees C, & 45 degrees C)
Petri plates (1-2 per student)
Plastic bags with twist ties
Sterile water
95% ethand
flame source

Web Resources
http://mt vernon.wsu.edu/path_team/Disease%20Gallery/dg87L.jpg (Carrot soft rot image)
http://edis.if as.ufl.edu/scripts/BODY_VH012
http://www .ces.uga.edu/pubcd/C809-w.htm
http://www.uga.edu/v egetable/carrot.html
http://www.nabt .org/sub/s tore/books/l bpp.asp (order form for Learning Biology with Plant Pathology from Nat. Association of Biology Teachers)

Safety

Duration
Varies depending upon activities chosen (2-10 days)

Procedure
A variety of experimental choices exist in investigating bacterial soft rot. Begin by preparing fresh nutrient agar test tube slants on Petri plate cultures of Erwinia carotovora. A helpful guide for this is the publication from NABT referenced in the web resources above. Some possible activities/investigations:

To Inoculate Vegetable Slices
Wash potato tubers, carrots, onions, and cut into slices about 7mm (1/4 inch) thick. Put about 1/8 inch of sterile water in a Petri dish that contains two disks of sterile filter paper and add the vegetable slices.

Make a slight cut in the center of each slice, and inoculate with a loopful of cells from the culture. Include a non-inoculated slice for each treatment used. Seal plates with strips of Saran Wrap or Parafilm.

To Inoculate Whole Vegetables

Carefully wash the surface of whole potato tubers, carrots, and onions. Sterilize a teasing needle by dipping in 95% ethanol and flaming until red-hot. Let the needle cool (this is very important), dip into a culture of Erwinia carotovora and then stab the potato 4-5 times with the needle. Repeat this 2-3 times in the same area of the potato. Follow the same procedure for the carrot and the onion. For controls, use the exact procedure, except dip the teasing needle into sterile water (instead of the bacterial culture) prior to stabbing the vegetables. Place inoculated vegetables onto a wet paper towel inside a plastic bag and seal tightly with a twist tie. Use a separate bag for each vegetable and separate bags for the controls (very important).

To Test Temperature Effects on Soft Rot

Inoculate the tissue slices of whole vegetables in a refrigerator, at room temperature, at 26 degrees C, and 45 degrees C if incubators are avalable. Different groups of students can choose one temperature.

To Determine Enzyme Activity and Soft Rot Development

The inoculated tissue slices should be checked starting at 48 hours whereas the whole vegetables should be checked beginning at five days after inoculation. For the tissue slices, test for tissue maceration (soft rot) by running a transfer loop evenly over the slice and/or by probing with a teasing needle. Do the same for the inoculated area on whole vegetables (be sure to use separate transfer loops or teasing needles for inoculated tissues and controls!). Continue to examine for 2-3 days and note the difference in rot produced on the different vegetables and between the different inoculation (temperature) conditions. Note the odor produced by inoculated, rotting tissue. Note: If inoculated vegetables in the bags are incubated for approximately two weeks at room temperature or 26 degrees C they will ÒmeltÓ and become almost totally liquefied due to the great amount of pectic enzyme activity.

To Demonstrate KochÕs Postulates

After students have observed the Òconstant associationÓ between tissues inoculated with Erwinia carotovora and soft rot symptoms, have them isolate the bacterium (using a sterile transfer loop) into pure culture on a nutrient agar plate (compare with the pure culture used for the original inoculations). After the pure culture has grown, use it to inoculate vegetables by the procedure described above (using the same vegetable source), observe the symptoms produced and then re-isolate the bacterium in pure culture (compare with original culture used to inoculate these vegetables.

Extension

Have students research KochÕs work with anthrax and discuss the similarities between his findings and the procedures from these activities.

Apply commercially purchased pectinase to vegetable samples and compare the results with what happened with the bacteria.

Student Questions (and answers)

1. Why are pectic polymers important in plants and what is their function? Pectic polymers are compounds that help provide support for cells. Pectins provide strength by helping to bind (cement) cells together into tissues.

2. How does Erwinia carotovora utilize the Òdigested Ó plant tissue as a food source? Plant pathogenic bacteria, such as Erwinia carotovora secrete enzymes such as pectinases that degrade plant cells and

tissues and then utilize the products of digestion by absorbing them.

3. Do other plant pathogens such as fungi and viruses produce cell wall-degrading enzymes? In addition to plant pathogenic bacteria, fungi also produce enzymes that are secreted into infected plant tissues and the products of enzymatic activity provide nutrients for the fungus. Viruses that infect plants do not produce enzymes.

4. What was the effect of temperature on the rate of the soft rot experiment? Why? Refrigeration thwarts rot development as does extremely high temperatures because it arrests growth of Erwinia carotovora and minimizes enzyme production. Room temperature and 26 degrees C are more conducive to bacterial growth and enzyme production. All plant pathogenic bacteria have an optimum temperature for growth and pathogenesis.

5. What do the plant materials (vegetables) used in this experiment all have in common? All of these vegetables consist of fleshy storage organs that are soft, contain abundant moisture, and are high in carbohydrates.

6. Why did the sliced tissues develop soft rot faster than the whole vegetable? The slices were already pre-wounded by cutting prior to inoculation and there were more bacteria per unit of tissue as compared with the whole vegetable.

7. What is the importance of KochÕs postulates? KochÕs postulates are utilized to prove the actual cause of a given plant disease. Without knowing the cause it is impossible to develop effective control measures for any disease.

Taken from Plant Pathology Teacher Workshop, 2001, University of Georgia Department of Plant Pathology.
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Student Sheet

Overview

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Questions
1. Why are pectic polymers important in plants and what is their function?
2. How does Erwinia carotovora utilize the Òdigested Ó plant tissue as a food source?
3. Do other plant pathogens such as fungi and viruses produce cell wall-degrading enzymes?
4. What was the effect of temperature on the rate of the soft rot experiment? Why?
5. What do the plant materials (vegetables) used in this experiment all have in common?
6. Why did the sliced tissues develop soft rot faster than the whole vegetable?
7. What is the importance of KochÕs postulates?