

“Root Nodulation” Microbes Fertilize Plants

Science in the Real World Microbes In Action

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Teacher Pages

At a Glance

Description

In this lab students will evaluate

Time Requirements

This lab will require

Curriculum Placement

This lab would fit well into a microbiology unit, a botany unit (plants as producers of antimicrobial agents) or relating to our human body in a study of our senses (aromas and sense of smell.)

Equipment

Autoclave or pressure cooker

Materials

- Hairy Vetch seeds; about 100 seeds/group. (1/4 lbs. bag available through Johnny's Selected Seeds Co., phone # 207-437-4301)
- Seed inoculant (*Rhizobium leguminosarum*) (also available through Johnny's Select Seeds Co.)
- Distilled water for watering the plants
- Graduated cylinder (50 ml or larger)
- 16 clear plastic beverage cups (8-10 ounce size) per group
- 8 quart bag of perlite or vermiculite (enough for about four groups)
- 2 large screw top bottles to hold fertilizer mixtures (empty 1 or 2 liter soda bottles will work)
- 1 Bottle of phosphorus and potassium ONLY fertilizer (the P and K source.)
Alaska brand "Mor Bloom" a 0-10-10 % N-P-K fertilizer is available in 1 quart bottles at most garden stores.)
- 1 Bottle of ammonium nitrate ONLY fertilizer (contains no phosphorus or potassium and only a Nitrogen source. A dry version was found referred to as "Nitrate of Soda" a 16-0-0 % N-P-K fertilizer which will become the nitrogen source.
- 1 500 ml beaker for mixing seeds and inoculant.
- 16 empty clear plastic soda/juice bottles to be cut at top and bottom to produce a tall cylinder to prop up the seedlings.
- 4 plastic spoons (for mixing substrate and water and for making holes to place the seeds) per group
- 1 Permanent marking pen for labeling the cups per group
- Plastic wrap to cover cups to promote fast germination and to prevent the substrate from drying out too quickly
- Area for plants to grow with adequate lighting

Root Nodulation: A Partnership in the Web of Life

Background

Nitrogen is an essential nutrient for all life on earth. The atmosphere is 80% nitrogen gas (N_2), therefore plants and animals are surrounded by this element and yet this is the element that most limits their growth. The problem is that plants and animals cannot use nitrogen gas (N_2) in this form. The old saying about the ocean, "water, water everywhere, and not a drop to drink," is appropriate for thinking of this phenomenon. Air containing mostly nitrogen surrounds them but none is useable. Plants and animals require the nitrogen in the correct form for their cells to absorb it and then chemically use it. Nitrogen gas (N_2) must first be changed to a nitrate compound (NO_3) and then to an ammonia compound (NH_4) before it can be put to use by plants. Plants then can metabolize (chemically use) the ammonia compound for making proteins and nucleic acids such as RNA and DNA. Proteins are used by the plant as structural proteins, plant pigments, plant hormones, etc. Nucleic acids act as information storage molecules. DNA stores all of the hereditary information and the RNA is involved in the making of proteins for the plant cells. Once the plant incorporates the nitrogen into their cells, then the animals can acquire the nitrogen by eating the plants.

??How do the plants acquire the nitrogen in the correct form??

Bacteria in the soil commonly convert nitrogen gas into ammonia, which then can be absorbed through the plants roots. Plants then build the important organic chemicals needed by the cells. Therefore, this process benefits all life forms ranking second to the process of photosynthesis. The plants are dependent on the bacteria. If they cannot get enough nitrogen, they will not grow to their potential. Farmers apply nitrogen fertilizers such as nitrates and ammonium to make sure that their plants have enough nitrogen to produce the crops.

Since Roman times, a group of plants called legumes (beans, peas, alfalfa, clover, etc) were known to accumulate favorable nitrogen in the soil. Legumes were used for crop rotation then and still are being used by farmers today. Theophrastus (370-285 B.C.) who is often referred to as the "Father of Botany", stated ".....beans are not a burdensome crop to the ground, they seem to manure it.....wherefore the people of Macedonia and Thessaly turn over the ground when it is in flower." In 1886 Hellriegel and Wilfarth demonstrated that legumes convert nitrogen gas in the atmosphere into compounds used by the plants due to their association with the presence of swellings or nodules on the legume root.

In 1888 Beijerinck, using Koch's postulates, isolated a root-nodule bacteria referred to as rhizobia. He was able to infect a legume to produce nitrogen- fixing root-nodules. Beijerinck's research was first to prove the known symbiotic relationship (partnership) between legume plants and some soil bacteria. In this association the plants provide a lump-like structure on the root for the bacteria to live and provide the bacteria with a carbon source used for energy . In return the plant receives the needed nitrogen compounds for metabolism of important compounds and healthy growth.

The symbiotic relationship, (partnership), in this case, is an association where both organisms benefit. This association is also referred to as mutualism or mutualistic symbiosis. Through evolution these partnerships become specialized between the bacteria and their host plant. Today it is known that the bacteria named *Rhizobium leguminosarum* enter the legume plant's root cells through an exchange of chemical signals that triggers the formation of the nodule. The bacteria then lives within the thick protective coating, supplied with all the carbon it needs and in return for this energy rich environment, the bacteria may fix hundreds of times more nitrogen than the free living bacteria in the soil.

Purpose

In this experiment, we will examine what it takes for root nodules to form on legume plants. We will consider the symbiotic relationship resulting from root nodules by observing the growth and appearance of the plants when exposed to *Rhizobium* as compared to the plants, which were not exposed to the *Rhizobium* bacteria.

Materials

Hairy Vetch seeds; about 100 seeds/group.
Culture of *Rhizobium leguminosarum*
Distilled water for watering the plants
Graduated cylinder (50 ml or larger)
16 clear plastic beverage cups (8-10 ounce size) per group
8 quart bag of perlite or vermiculite (enough for about four groups)
2 large screw top bottles to hold fertilizer mixtures
1 Bottle of phosphorus and potassium ONLY fertilizer
1 Bottle of ammonium nitrate ONLY fertilizer
1- 500 ml beaker for mixing seeds and inoculant.
16 empty clear plastic soda/juice bottles
4 plastic spoons
Permanent marking pen
Plastic wrap

Overview:

Two variables will be tested for nodulation formation: nitrogen fertilizer and nitrogen fixing bacteria. Four groups of plants will be prepared by a team. Each team will prepare a set of cups marked +nitrogen +bacteria and a set marked + nitrogen - bacteria (4 cups of each type.) Other team members will prepare a set marked - nitrogen + bacteria, and a set called -nitrogen - bacteria (again 4 cups of each type.)

Procedure-Day One

Preparing the soil:

1. Fill the cups 2/3 full with perlite or vermiculite and add sufficient water to moisten thoroughly (approximately 50 plus mL. of distilled water.)
2. Stir well with plastic spoon to be sure to moisten all the potting mixture (Mixture

must be very wet yet the seeds must not be submerged in water for they will suffocate!).

3. Label the four groups of cups that will be prepared by the team. The team will prepare a set of 4 cups marked "+nitrogen +bacteria" and a set of 4 cups marked "+nitrogen -bacteria," a set of 4 cups marked "-nitrogen +bacteria," and a set of 4 cups marked "-nitrogen-bacteria." There will be a total of 16 cups prepared by the team.
4. Be sure to put the initials of your team members along the base of each cup and also the date the cups were planted.
5. Space 5-6 holes in the substrate about 1 cm deep with the spoon handle to plant the seeds.

Preparing the seed:

1. The seeds for plants grown without bacteria can be planted directly from the package into the holes of the cups marked "-bacteria." (approximately 50 seeds.)
2. Make a hole about 1 cm deep into perlite, push the perlite over the seed. When all cups without inoculant are planted, cover with plastic wrap and place in a warm sunny place.

Note: If a station is not set up for inoculated seeds, then it is best to plant the seeds without inoculant first to prevent accidental exposure to the bacteria.

3. First count out seeds for all 8 cups (approximately 50 seeds). Figure 5-6 seeds per cup and place in a beaker.
4. Add a few drops of water and then a 1/2 teaspoon of inoculant to coat the seeds with a soil-like mixture (seeds will be coated in black inoculant.)
5. Plant the seeds in the holes of cups labeled "+bacteria." Make sure that some of the inoculant is allowed into the hole with the seed.
6. Cover with plastic wrap and place in sunny warm location.

Procedure: Day Two and for further observations

Observations, water and fertilizer:

1. Make sure the cups are checked for moisture daily to determine when to add water and/or fertilizer.
2. Make a record of how fast the seedlings germinate in each cup and the amount of growth (height) can be measured in mm/cm with each observation.
3. Also make note any differences in the size, growth rate, color of leaves, or overall health of the plant. (Pale green leaves or yellowing leaves often are a sign that a plant is lacking something)
4. Add fertilizer once all or most of the seeds have germinated.
5. Thin the seeds to the three or four healthiest plants per cup. Add 20 ml of the proper fertilizer solution.
6. Do not add fertilizer daily...only once every 3-4 weeks is recommended.) Add enough water daily to maintain good moisture in the substrate but not enough to

submerge and suffocate the seeds. The seeds must "breathe" in order to carry on cell respiration!

7. After initial fertilization, only add water on a need be basis and fertilize using 20 ml of fertilizer solution every 3 weeks for the duration of the experiment.

Looking for Nodules:

1. After about 1 month of growth, carefully pull up one of the vetch plants grabbing low on the shoot so that the roots come up with the plant.
2. Dunk the root system in a beaker of tap water to clean the roots and look for nodules. You should find them on the main tap root near the surface but you can also find them lower down on the root system.
3. Record the number of nodules per plant , keeping track of what type of treatment was received by the plant during the experiment ("+bacteria" or + nitrogen or both)

Optional Gram Stain for Nitrogen Fixing Bacteria:(requires a microscope with oil immersion lens)

1. Clean the root well. Remove a nodule using a razor blade and place on a microscope slide.
2. Using the edge of another slide, smash the nodule well. Add 1 drop of distilled water and spread around the slide.
3. Pass the slide over the flame of a Bunsen burner quickly to dry.
4. Over a sink, add 1 drop of crystal violet stain and let sit for 30 seconds.
5. Rinse crystal violet stain with distilled water and let dry (The slide should be clear with tiny purple specks on it.)
6. Under 100 X oil immersion lens look for structures that you do not see on the uninoculated root tissue.

Name _____

Analysis Questions:

1. What are the independent variables?
2. What are the dependent variables?
3. What are the constants?
4. Which set of cups are the controls?
5. Create a table to display the data collected over a series of weeks.
6. Did you notice any differences in how well the plants grew? Or did all the sets of plants look equally healthy? Explain your findings.
7. Can you generalize about how you think plants get their nutrition? In other words, where do plants get the nutrients that they need to grow?
8. After studying legume plants, can you think of any ways that bacteria are beneficial to all of us?
9. Can you explain the relationship between legumes and Rhizobium bacteria?
10. Why do you think that we used perlite or vermiculite and not potting soil?

Optional Extensions:

1. Draw a picture of the stained nodule versus the tissue lacking a nodule. Label the difference.
2. Find out how plants use the nitrogen, phosphorus and potassium found in most fertilizers. What are the benefits of adding these chemicals to the soil for the plant?
3. Report on one other symbiotic relationship that deals with a partnership between bacteria and another organism.

Teachers Guide

Instructional Objectives

At the end of this activity, the students should be able to:

1. Demonstrate the following laboratory skills:
 - a. Comparing and contrasting
 - b. Following the directions to complete a variety of tests
 - c. Constructing a graph
 2. Demonstrate the methods of scientific inquiry by:
 - a. Perform an experiment according to given directions
 - b. Gathering and organizing data
 - c. Analyzing data
 - d. Applying understanding of a specific principle to a more general purpose
- Using perlite, this experiment can also be an introduction to hydroponic plant growth. You need to discuss the fact that perlite is lava rock and supplies not nutrients.
 - This lab is an excellent way to introduce the nitrogen cycle as well.
 - This lab is quite useable for the block schedule. The only caution would be to water sufficiently when students leave class on Thursday and not return until Monday of the following week. A plastic wrap lightly over the plants help to retard evaporation.
 - A team of 4 students would result in each student preparing 4 pots each. They would work together to decide who does each set. I would have them record data on their 4 pots and snip the root wad at the lower end of the stem and preserve in 10% ethanol so the team can look at all the root development later when trying to analyze their results. This is an optional suggestion in the lab write up.
 - Your initial investment in fertilizers will cost you about \$12.00 but you will be able to use it for years. You can also recycle the perlite into potting soil and reuse for planting.
 - 'Bottle Biology' can be used to make clear growing containers out of recycled plastic soda and juice bottles. This would save on purchasing so many clear cups. Remember that foam cups are cheaper and work well however one cannot see the root development through the sides as one can in the clear containers. Also the clear 16-20 ounce size clear plastic bottles were used to surround the seedlings and keep them from falling over in their initial growth stages. One could

set up cutting stations to make clear plastic growing containers out of bottoms of soda bottles. Another station could be set up to make the cylinders to prop up the seedlings.

Another method was to make cylinders out of clear transparencies to sit inside the cups and support the seedlings.

- Allowing the vetch plants to climb string or sticks keeps them from falling over. Mine ended up climbing the venetian blinds until they were ready to be pulled.

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