SCIENCE IN THE REAL WORLD

Microbes in Action

They're Everywhere!

A Food Preservation Lab

Science in the Real World

Microbes In Action

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At a Glance

Description
This lesson consists of two laboratory exercises in which students explore microbial growth in common foods and methods of food preservation. In “Hello!...Anybody There?”, plain non-nutrient agar is enriched with various types of food and then observed over a period of several days for bacterial and fungal growth. “There’s A Fungus Among Us” investigates the effectiveness of salt, sugar or chemicals as preservatives.

Time Requirements
Each of these lab activities can be set up in one class period. You will need a few minutes for observation on selected days for 5-10 days. You will see results in 5-7 days if the plates are stored at room temperature, or sooner if incubated at 30°-32°C.

Curriculum Placement
The exercise can be used in any general biology course in conjunction with the following topics: microbiology, health and nutrition, application of biology to everyday life, diversity among living things, characteristics of mold and bacteria, and the role of osmotic pressure in microbial growth.

Materials
Bunsen burner
inoculating loops
permanent markers
plastic teaspoons
sterile empty petri plates
parafilm or masking tape
quick oats
sucrose
assorted food items
plain agar
Penicillium notatum culture (plates)
Bacillus subtilis culture (plates)
Potato Dextrose agar plates (for stock culture)
Nutrient agar plates (for stock culture)
droppers
toothpicks
propionic acid
sorbic acid
sodium nitrite
salt

Background

Food preservation is necessary for storing food safely and for maintaining food palatability. Through the ages people have developed various preservation methods, some of which are still used today.

One of the earliest methods of food preservation was drying. Early methods of drying included using the sun's heat or warm winds to dehydrate foods. There is evidence that early humans dried strips of meat, fish, vegetables, and fruits using this method. Later, people developed heat chambers. Smoked meats and fish may have resulted from such drying methods. Smoking dries the food and adds a film of phenolic, cresylic, and aldehyde compounds on the surface that hardens to form a crust that inhibits food spoilage.

People of temperate and arctic regions have used natural refrigeration during appropriate seasons. Cool recesses in the earth have also been used to store food. It was not until 1877 that mechanical refrigeration and freezing was developed so that frozen meat could be shipped from Australia to France and England. In 1929, Clarence Birdseye developed commercial freezing, which is very popular today.

Canning was developed in 1809 by Nicolas Appert who used wide mouth glass bottles that were carefully heated in boiling water. This method not only kills microbes but seals the jars to prevent further contamination. The method we now call pasteurization was developed by Louis Pasteur. In this process, food is heated to 63°C for a short period of time to kill most, but not all, microbes.

Chemical preservatives such as salt and sugar at high concentrations work by reducing the amount of water available to the microbial cells. This alters the osmotic pressure in the cell which ultimately decreases metabolic activity. This then decreases microbial growth. There are groups of microbes that can withstand harsh environments. Osmophilic microbes have evolved to withstand extremely high concentrations of salt and sugar. Halophilic microbes can withstand concentrations of 25-30% salt while saccharophilic microbes survive sugar concentrations of 50-75%. The average jelly or jam is 67% sugar. Since fungi are more resistant to low external water concentration than are bacteria, they can often survive in high sugar or salt environments, while bacteria do not. Various organic acids are added to certain foods to inhibit microbial growth. Propionic acid and sorbic acid interfere with cell metabolism. Another type of preservative, the nitrates and nitrites, are often used to preserve meats.

Following is a list of some of the more common food preservatives and their uses. Food preservatives are utilized to prevent spoilage and act as either antimicrobials or antioxidants or both. Antioxidants keep food from browning, becoming rancid or developing black spots. They suppress the chemical reaction that occurs when foods combine with oxygen. Preservatives also act to minimize damage to some essential amino acids and to prevent the loss of some vitamins.

NITRATES

- serve as antimicrobials in meat to inhibit the growth of bacterial spores that cause botulism
- preserve flavoring and fix color in a number of red meats, poultry and fish products

BHA and BHT

- slow the development of off-flavors, odors and color changes from oxidation in many foods high in fats and oils
- are also used as preservatives in dry foods such as cereals

SULFITES

- serve as antioxidants to prevent or reduce discoloration of light-colored fruits and vegetables, such as dried apples and dehydrated potatoes
- are used in wine-making after yeast fermentation to inhibit microbial growth
- are used in bleaching of food starches and as preventatives against rust and scale in boiler water used in making steam that will come in contact with food

SORBIC ACID

- is used in acid-type foods such as hard cheeses, figs, syrups, salad dressings, jellies and cakes to prevent or delay microbial growth.

PROPIONIC ACID

- is used in acid-type foods such as breads, cakes and some cheeses to prevent or delay microbial growth.

Name _____

Date _____

Hello! . . . Anybody There?

Microbes in Food

Purpose

You will observe microbial growth on foods.

Background

The nutrients in food support a variety of living organisms, including many bacteria and fungi. The products of microbial growth alter the smell, taste and texture of many foods, often making them unpalatable, or sometimes dangerous, to humans. Microorganisms can also cause changes that make food more palatable, such as the conversion of milk to cheese or cucumbers to pickles.

Food may become contaminated with microorganisms in a variety of ways. Some bacteria and fungi come from the soil used to grow food. Other foods such as milk may become contaminated during harvesting or storage. Some foods become contaminated during handling, as in the butchering of meat. There are microorganisms everywhere and it is impossible to prevent them from contaminating food.

Since we cannot prevent microorganisms from finding food we would like to eat, we have developed many ways to protect the food from microbial growth. Microorganisms are made of cells that are similar in many ways to animal or plant cells. If we treat food in such a way that it prevents or slows cell metabolism, microbial cells (bacteria, protozoa, or fungi) will not be able to grow well. If the microbes don't grow well, the food does not spoil, or does so much more slowly.

Materials- (per group of 4)

5 sterile empty petri plates
assorted food items
125 ml of molten agar at 50° C
droppers
parafilm or masking tape
4 plastic teaspoons

Procedure

After reading the procedure, your group should determine how to prepare a control plate for this experiment. Discuss your plan with your teacher before preparing your plate. Record your procedure in the space provided in the Data and Analysis section.

1. Work in groups of 4. Each student should use a **different** food item. Select your food item from those available.

2. Each student in your group needs one petri plate. The petri plate is sterile. DO NOT open it until you are ready to use it. On the bottom of the petri plate (the smaller diameter half) write your name, food item, and date. Write in small letters near the edge of the plate bottom.

3. Carefully lift the lid of your petri plate only long enough to add your food item (approximately **one heaping teaspoon**). Replace the lid. If the item is a liquid you can simply pour it into the petri plate; if it is a solid like a cracker, crumble the cracker then add it to the plate; if the item is solid like a peach, place several small slices of the item in the plate.

4. Carefully lift the lid of your petri plate enough to pour warm agar over your food item until the petri plate is approximately half full (20-25 ml). Replace the lid. **Gently** swirl the petri plate on the countertop in a figure-eight motion to evenly distribute the agar and food item.

5. Prepare your control plate.

6. When the agar is solid, tape the petri plate around the outside edge using parafilm so that you are still able to see the surface of the agar.

7. Predict which foods will show the greatest and least growth of microbial contaminants. Explain the reason for your prediction. Record this in the space provided in the Data and Analysis section on the next page (see number 3).

8. Over the course of the next week, on days selected by your teacher, you will make observations of your group's plates. Construct a data table in the Data and Analysis section on the next page to record your group's observations(see number 1).

DO NOT OPEN THE PETRI PLATE WHILE MAKING OBSERVATIONS!

Name _____

Date _____

Hello! . . . Anybody There?

Data and Analysis

1. Construct a data table on the back of your paper to record your group's data.

2. Describe your group's control and explain why it is a control.

_____

_____

_____

3. What is your prediction about which foods will show the greatest or least microbial growth?

_____

_____

4. On the back of this sheet of paper, construct a data table to record **Class** data.
(by collecting information from the other groups in your class)

5. Rank the food items from the most contaminated to least contaminated.

_____

_____

_____

_____

6. What might be the source of contamination?_____

6. Should you be concerned about these contaminants?_____ Why or why not?

_____

There's A Fungus Among Us

Food Preservation

Purpose

You will observe the effect of various sugar and salt concentrations and various chemicals on the growth of *Bacillus subtilis* (a bacteria) and *Penicillium notatum* (a fungus), and you will determine the effectiveness of these compounds as preservatives.

Materials - per team of 4

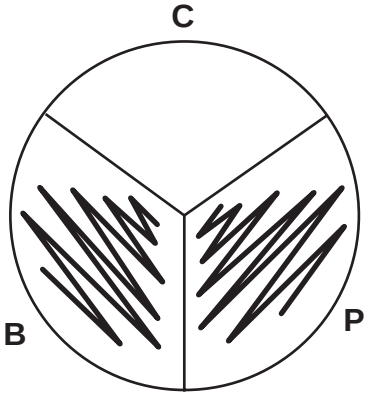
Penicillium notatum culture on a plate
Bacillus subtilis culture on a plate
1/4 cup quick oats
plastic spoon
4 empty sterile petri plates
100 ml of molten agar at 50° C
30 g of sugar **OR** 10 g salt **OR** 3 ml each of distilled water,
3% propionic acid, 3% sorbic acid and 3% sodium nitrite
4 droppers
toothpicks
inoculating loop (optional)
parafilm or masking tape
graduated cylinder (10 ml)

Procedure

1. Work in groups of 4. Each group should pick one type of food preservative (the variable) - salt, sugar or chemical.

2. Each student in the group needs one petri plate. The petri plate is sterile-DO NOT open it until you are ready to use it. On the bottom of the petri plate (the half with the smaller diameter) write your name and date. Divide the plate into three sections labeling one third **C** for control, the other **B** for *Bacillus* and the third **P** for *Penicillium*. Add the name of the preservative series your group chose to use (salt, sugar, chemical) as well as the % concentration (if applicable). See Figure 1. on next page.

Figure 1- Streaks of cultures of *Penicillium* and *Bacillus*



3. Each student in the group should add a level teaspoon of oats to his or her petri plate. Be careful to avoid contamination by lifting the lid just enough to add the oats. In this mixture, the oats provide the nutrients and the agar provides the semi-solid gel that holds the water.

4. After choosing your variable, refer to the chart below and add the correct amount of sugar, salt or chemical to your petri plate. (20 drops = 1 ml)

Plate	Salt		Sugar		Chemicals	
	Final %	Amount	Final %	Amount	Final %	Amount
1	0%	0	0%	0	0	3 ml distilled water
2	5%	1.25 g	15%	3.75 g	0.4%	3 ml sorbic acid (provided at 3%)
3	10%	2.50 g	30%	7.50 g	0.4%	3 ml propionic acid (provided at 3%)
4	20%	5.00 g	60%	15.0 g	0.4%	3 ml sodium nitrite (provided at 3%)

5. Add warm agar to your petri plate until the plate is approximately half full (about 25 ml) and mix contents together with a toothpick. Do not move the plate until the agar solidifies. This will take about 20 minutes.

6. Streak the solidified agar in your petri plate with *Penicillium notatum* and *Bacillus subtilis* in the appropriate sections. Using sterile technique, remove a small amount of the microbial growth from the stock plate with an inoculating loop or sterile toothpick. Transfer this material to your petri plate and streak as shown in Figure 1.

7. Tape the petri plate around the outside edge using parafilm or tape, so that you are able to see the surface of the agar medium.

8. Over the course of the next week, on days selected by your teacher, you will observe your group's petri plates. Construct a data table to record your group's observations (see item #1 under Data and Analysis).

DO NOT OPEN THE PLATE WHILE MAKING OBSERVATIONS!

Name _____

Date _____

There's A Fungus Among Us
Data and Analysis

1. On the back of this sheet of paper, construct a data table to record class data. Devise a scale to record the following observations:

no growth minimal growth some growth substantial growth

2. Describe the appearance of the *Bacillus subtilis* and *Penicillium notatum* growth.

_____

_____

_____

3. Using the class data, compare the growth of the bacterium *Bacillus subtilis* and the fungus *Penicillium notatum* based on their tolerances to the substances used in the activity.

_____

_____

_____

4. How does high salt concentration or high sugar concentration affect the cellular functions of the microbes used in this activity? Include a diagram of a cell to illustrate your explanation.

_____

_____

_____

5. Using class data identify several methods by which foods can be preserved. Give an example of a food preserved by each method.

_____

_____

_____

6. Refer to the data from “Hello!...Anybody There?”. Using the knowledge you now have, explain any of the differences in microbial growth you observed. Check the original packages for these foods. Is there any ingredient(s) listed that validates your explanation?

_____

_____

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_____

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_____

7. At home this evening, go to your pantry shelves and/or refrigerator and look at the labels of 3 food containers. For each one, list the brand name, the food and any chemicals that you think serve as preservatives.

_____

_____

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_____

_____

_____

_____

_____

_____

_____

_____

8. Expanding on the information learned in this lab, fill in the following chart. Use a check to select where you would store the following food items: S= shelf, R= refrigerator and
   F= freezer. In the last column, explain your answer.

Food Item	S	R	F	Why?
Cheerios™				
plums				
prunes				
cucumbers				
pickles				
fresh peaches				
canned peaches				
roast beef				
beef jerky				
apples				
applesauce				
grapes				
grape jelly				
raisins				
flour				

9. In this lab you investigated the effectiveness of salt, sugar and assorted chemicals as preservatives. Design an experiment that tests the effectiveness of any other method of food preservation. This could include canning, drying, etc. Include a protocol, materials list and control.

_____

_____

_____

_____

_____

Teacher Pages
Sources of Supplies

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

Description	Stock Number	Quantity
Penicillium notatum	K3-15-6155	1 plate
Bacillus subtilis	K3-15-4921	1 plate
petri plates (500 per case)	K3-74-1350	1 case
propionic acid	K3-88-4938	500 ml

Sigma Chemical Co.
P.O. Box 14508
St. Louis, MO. 63178

Description	Stock Number	Quantity
agar	A 7002	100 g
nutrient agar	N 4019	250 g
potato-dextrose agar	P 2182	250 g
sorbic acid	S 1751	100 g
sodium nitrite	S 2252	500 g

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 according to given directions
    d. gathering and organizing data
    e. analyzing data
    f. developing further investigations

2. demonstrate the following laboratory skills:
    a. pour agar plates
    b. use sterile technique
    c. use an inoculating loop to transfer bacteria
    d. create a control
    e. construct a data table

3. demonstrate an understanding of:
    a. microbes
        I. the role of osmotic pressure in cell membrane transport
        II. distinguishing physical characteristics of microbial colonies
        III. metabolic requirements for microbial growth
        IV. environmental conditions needed for growth
        V. the prevalence of microorganisms in the environment
    b. food preservation
        I. metabolic basis of inhibition of microbial growth
        II. common methods used in the food industry
        III. effectiveness of these methods as inhibitors of bacteria and fungi

Preparations

"Hello! . . . Anybody There?"

Materials (per group of 4)
5 sterile petri plates
assorted food items
100 ml of plain molten agar at 50°C
dropper(s)
parafilm or masking tape
4 plastic teaspoons

"There's A Fungus AmongUs"

Materials (per group of 4)
Penicillium notatum and *Bacillus subtilis* cultures
1/4 cup quick oats
plastic spoon
4 petri plates
100 ml of plain molten agar at 50° C
approximately 30 g of sugar OR 10 g salt
4 droppers
toothpicks
graduated cylinder (10 ml)- optional
3 ml each of distilled water, 3% propionic acid, 3% sorbic acid and 3% sodium nitrite

Cultures

***Penicillium notatum*:**

Three days before "There's A Fungus Among Us", streak potato-dextrose agar plates with *Penicillium notatum* from a stock culture and incubate at 30-32° C. An inoculated plate should be sufficient to provide *Penicillium notatum* for six or more student groups. *Penicillium notatum* plates will keep in the refrigerator. Long term storage of *Penicillium notatum* should not be a problem once green spores are produced. Wrap plate in plastic wrap to prevent moisture loss. If incubator is not available, start culturing 5-7 days in advance at room temperature. After growth is visible, store in refrigerator.

***Bacillus subtilis*:**

24 hours prior to lab streak nutrient agar plates with *Bacillus subtilis* and incubate at 30-37 °C. If good growth is visible, store in refrigerator after 18-24 hours. *Bacillus subtilis* cultures will lyse over time in the refrigerator. Reculture weekly or every other week to maintain a healthy stock.

Agar Preparation

Agar:

Prepare agar by dissolving 15 g of plain agar (not nutrient agar) in 1000 ml of tap or distilled water. Heat to boiling with frequent stirring. Autoclaving is not necessary, unless you plan to store this for more than a few days. The agar can be prepared in advance. Prior to each lab liquefy agar by heating in a boiling water bath (this may take 30-60 minutes). Melting agar in a microwave often results in the agar exploding all over the inside of the oven unless your container is MUCH larger than the amount of agar! You can maintain melted agar in a warm water bath (50° C).

Nutrient Agar Plates:

Nutrient agar is prepared by dissolving 23 g of nutrient agar in 1000 ml of distilled water (although tap water would work). Autoclave for 15 minutes at 15 psi. After cooling to about 50°C, pour approximately 20-25 ml into each petri plate.

Potato-Dextrose Agar Plates:

Potato-dextrose agar is prepared by adding 39 g of potato-dextrose agar to 1000 ml of distilled water. Autoclave for 15 minutes at 15 psi. After cooling to about 50°C, pour approximately 20-25 ml into each petri plate. Sabouraud-dextrose agar can be substituted for potato-dextrose agar, but must be prepared according to package instructions.

“Hello!. . .Anybody There?” Answer Key

1. Construct a data table to record your group’s data.

   Group data will vary.

2. Describe your group’s control and explain why it is a control.

   Control: Agar in a plate with no food added.

3. What is your prediction about which foods will show the greatest or least microbial growth?

   Predictions will vary.

4. Construct a data table to record Class data.

   Class data will vary.

5. Rank the food items from the most contaminated to least contaminated.

   Order will vary but foods with preservatives should be lower on the list.

6. What might be the source of contamination?

   Contaminants are naturally found on many foods, some come from the soil while some arrive by insects or other organisms, others are carried by the air. It is possible that contaminants are accidentally added in food processing, transport or kitchen preparation.

7. Should you be concerned about these contaminants? Why or why not?

   Generally there is little concern with small numbers of contaminants because the enzymes in the mouth and stomach and the acidic environment of the stomach destroy them. Also boiling, baking, or microwaving will destroy most microbes.

"There's A Fungus Among Us" Answer Key

1. Construct a data table to record class data. Devise a scale to record the following observations: no growth minimal growth some growth substantial growth

   Data tables will vary.

2. Describe the appearance of the Bacillus subtilis and Penicillium notatum growth.

   Bacillus subtilis appears as an opaque, white, and waxy growth. Penicillium notatum appears initially as a white, fuzzy growth which turns green as it matures and spores are produced.

3. Using the class data, compare the bacterium Bacillus subtilis and the fungus Penicillium notatum based on their tolerances to the substances used in the activity.

   Generally Bacillus subtilis is less tolerant of high sucrose and salt concentrations than is Penicillium notatum. Penicillium notatum growth is reduced at 20% salt and 60% sucrose but does show more growth than Bacillus subtilis at these concentrations. Neither microbe is tolerant of propionic acid, but both tolerate sorbic acid. Penicillium notatum tolerates sodium nitrite while Bacillus subtilis growth is suppressed.

4. How does high salt concentration or high sugar concentration affect the cellular functions of the microbes used in this activity? Include a diagram of a cell to illustrate your explanation.

   A high sucrose or salt concentration places a microbe into a hypertonic solution. Since the water concentration is less outside the cell than inside the cell, water will move by osmosis out of the cell and the cell will shrink (plasmolysis). The cell is dehydrated.

5. Using class data identify several methods by which foods can be preserved. Give an example of a food preserved by each method.

   Food can be preserved by placing the food in high salt concentrations (e.g. dill pickles), placing the food in high sugar concentrations (e.g. jellies) or adding a food preservative to nearly any processed food.

6. Refer to the data from "Hello!...Anybody There?". Using the knowledge you now have, explain any of the differences in microbial growth you observed. Check the original packages for these foods. Is there any ingredient(s) listed that validates your explanation?

   Answers will vary but look for the presence of salt, sugar or other chemical preservatives.

7. At home this evening, go to your pantry shelves and/or refrigerator and look at the labels of 3 food containers. For each one, list the brand name, the food and any chemicals that you think serve as preservatives.

   Answers will vary but look for examples such as: Smuckers/ Strawberry Jam/ sugar

8. Expanding on the information learned in this lab, fill in the following chart. Use a check to select where you would store the following food items: S= shelf, R= refrigerator and F= freezer. In the last column, explain your answer.

Food Item	S	R	F	Why?
Cheerios™	X			dehydration- microbes need water to grow
plums		X		lowers temp to slow microbial growth in the favorable environment of the fruit
prunes	X			dehydration- microbes need water to grow
cucumbers		X		lowers temp to slow microbial growth in the favorable environment of the vegetable
pickles	X			*acidic - preservative*
fresh peaches		X		*lowers temp to slow microbial growth in the favorable environment of the fruit*
canned peaches	X			*high temperature kills microbes*
roast beef		X	X	*lowers temp to slow/stop microbial growth in the favorable environment of the meat*
beef jerky	X			*dehydration- microbes need water to grow*
apples		X		*lowers temp to slow microbial growth in the favorable environment of the fruit*
applesauce	X			*high temperature kills microbes*
grapes		X		*lowers temp to slow microbial growth in the favorable environment of the fruit*
grape jelly	X			*high concentration of sugar- preservative (bacteria) lowers temp to slow fungal growth*
raisins	X			*dehydration- microbes need water to grow*
flour	X			*dehydration- microbes need water to grow*

9. In this lab you investigated the effectiveness of salt, sugar and assorted chemicals as preservatives. Design an experiment that tests the effectiveness of any other method of food preservation. This could include canning, drying, etc. Include a protocol, materials list and control.

Answers will vary.

Teacher Hints & Troubleshooting

1. Provide a variety of food items for students to select for “Hello!...Anybody There?” A large variety of food items support the growth of microbes. They range from fresh produce and meats to canned products and dried foods. Crackers, potato chips, baked goods, fresh fruits, vegetables, and cereals provide interesting results.

2. A comparison of a fresh food to its canned form usually produces a dramatic result and will add to the discussion. A measurable difference between “lite” and heavy syrup fruits was not observed.

3. Sterile technique needs to be reinforced. In the series of plates with varying sucrose solutions, sterile technique is very important because these plates are very easily contaminated by airborne molds.

4. In both labs, seal the plates to prevent the release of mold into the room if the petri plate is opened. Some students may be allergic to mold.

5. If a choice needs to be made between running the sucrose or salt series, select the salt series as it provides more dramatic results.

6. If necessary, discuss and demonstrate transfer of bacteria with an inoculating loop or sterile toothpick. Include precautions to minimize contamination of cultures.

7. Extensions of these activities include making sauerkraut or using a food dehydrating appliance to dry your own food.

Dear Parents,

Have you ever wondered why your cereal can sit on the shelf for months at a time but try it with chicken and you will never forget the results? And why is it that chicken in the freezer is fine for weeks or even months but not if you store it in the refrigerator? Why is fresh beef dated and yet beef jerky can sit on the counter of the “quicky mart” for years?

Maybe these questions are not at the top of your “Must Answer” list, but your children will be wrestling with these questions and more as they complete the lab experiments planned for them this week.

Check with them periodically to see what they have learned. And keep your pantry door opened! They will be looking at lots of labels in the days to come!

As always, you are welcome to come and visit to see “What’s Cooking” in the science labs!