Microbe Experimentation

Wyoming Science, Language Arts, Health

Objectives:

- Formulate a question about microorganisms that can be answered with a student experiment.
- Develop a hypothesis for a question about microorganisms based on observations and prior knowledge.
- Plan and carry out an investigation on microorganisms.
- Display results in an appropriate format (e.g., graphs, tables, diagrams).
- Prepare a written summary or conclusion to describe the results in terms of the hypothesis for the investigation on microorganisms.

Background:

Yogurt

What makes the milk thicken to make yogurt? During biblical times, people the in Middle East discovered yogurt. They found that when milk was left in a warm place, it thickened and developed a different, tart flavor. More importantly, it kept better than fresh milk. It was centuries later that scientists discovered that tiny bacteria made yogurt. Most yogurts are made from either lactobacillus bulgaricus or streptococcus thermophilus. Once the bacteria is added to milk, the bacteria consumes the milk sugars and undergoes fermentation, much like the yeast in bread. The benefit of having a fermented milk product is that so much acid is produced by these organisms that few other potentially harmful microorganisms can grow in this acid yogurt environment.

Not all processors process yogurt the same. Live active cultures will be necessary for this bacteria experiment. Making yogurt in the classroom is best done as a demonstration—unless you can get several small coolers for each group or team of students.

Sour Milk

Pasteurization is a heat treatment performed at the processing plant which destroys harmful bacteria without affecting the quality of the milk. Milk may be pasteurized using a low heat method (63°C, 145°F for 30 minutes) or a high heat method (72°C, 162° F for 15 seconds). Pasteurization does not kill all bacteria contained in raw milk, but it does kill those pathogens that may cause disease.

Standards

Science

Life Systems: 1.3, 1.6

Earth, Space, & Physical Systems: 1.1

Science As Inquiry: 2.1, 2.2, 2.3, 2.4, 2.5

Language Arts

Writing: 2.1A, 2.1C, 2.1D

Health

Health Promotion & Disease Prevention: 1.6

Influence of Culture, Media, & Technology: 4.1

Materials

Activity 1:

How to make yogurt

- 1-3/4 cups powdered milk
- 4 cups of warm water
- 1/3 cup of plain yogurt
- 5 one-cup containers w/lids
- Mixing container
- Whisk
- Cooler
- Fruit topping or pie filling
- Spoons
- Litmus paper
- Materials cont. on pg. 2

Estimated Time

3- (30) Minute Sections

Grades 5-6

materials:

Activity 2: Souring Milk

Teams of 3-4 Students

- 3 to 6 test-tube racks, depending on the number of teams. Teams can share test-tube racks.
- Refrigerator with freezer compartment, if possible
- 60 ml of pasteurized, whole milk (10 ml/test tube)
- 60 ml of ultra high temperature (shelf stable, UHT)
- 19 6th Grade Teacher Resource Book - Microorganisms in the Macrocosm
- 6 sterile test tubes
- 6 sterile test-tube caps or aluminum foil to cover the test tubes
- Two sterile 10 ml pipettes
- One or two sterile 5 ml pipettes or eye droppers
- 20 Sterile plastic pipettes
- Methylene blue 1% (to be diluted)
- Permanent marker to label test tubes
- Blue's the Clue Data Table

Bacteria that remain after pasteurization eventually cause milk to sour (spoil). Pasteurization also inactivates enzymes in the milk and destroys yeasts, molds, and other bacteria. Bacterial populations in milk are a direct indication of milk quality. Processing plants check the milk before they load it into a truck, again before the truck is unloaded at the processing plant, in the storage tank at the processing plant, before it is pasteurized, and after it is pasteurized. Milk lots are also tested daily for 10 days after they are bottled. There are two tests used primarily. The first test checks the concentration of microorganisms in raw and pasteurized milk. The second test detects viable and dead microorganisms.

In both pasteurized and raw milk, various microorganisms succeed one another as the chemical environment of the milk changes. The microbes themselves bring about these changes. The stages of microbial growth are Streptococcus, then Lactobacillus, then yeasts and molds, and finally Bacillus.

Streptococci convert the milk sugar (lactose) to lactic acid. The acidity of the milk increases to the point where further streptococci growth is inhibited. Lactobacilli then begin to grow and convert the remaining lactose into lactic acid. Acidity increases further until Lactobacilli growth is suppressed. The lactic acid sours the milk and curdles (coagulates) the milk protein. Yeasts and molds grow well in this acid environment, and they convert acid into nonacid products. Finally, bacilli multiply in the environment where protein is the only nutrient available.

Bacilli convert protein into ammonia products, and the pH rises. These bacteria also digest the remaining protein through enzymatic action. The odor of spoiled milk becomes apparent once this has happened. Microbial activity causes changes in the pH of the milk. Fluctuations in pH are due to fermentation and putrefaction (decomposition) process.

UHT or "ultra high pressure" treated milk is milk that is "ultra" pasteurized, making the milk sterile. Ultra high temperature, higher than pasteurization, and pressure is applied to the milk resulting in a sterile product.

Activity Procedures:

Activity 1: How to make yogurt?

Before you begin, make sure you have all the necessary ingredients:

- containers with lids (you can use previously used yogurt containers, or purchase 2 oz. cups with lids from any restaurant supply store or possibly your school cafeteria),
- a small insulated "cooler" that will be used as the incubator. Small "lunch tote" coolers work well and many come with a container that you can fill with hot water that will surely keep your yogurt warm for the 6-8 hours it will need to incubate.

Be sure to use a plain yogurt that contains active cultures. Taste the plain yogurt you plan to use as the starter culture. Your yogurt will have the same taste (Dannon works well). Making yogurt in your classroom is easy, and really "fool proof" if you follow the recipe and procedures.

Fool-Proof Yogurt

Mix in a deep mixing bowl:

- 1-3/4 cups powdered milk
- 4 cups very warm water (between 43-51°C, 110-125°F)
- 1/3 cup plain yogurt with active culture and no additives. (The yogurt will only be as good as the starter you use.)

1. Whisk the first two ingredients until dissolved.
2. Add the yogurt and whisk until most of the clumps are dissolved.
3. Work quickly, you don't want the mixture to cool.
4. Pour the mixture into the cups.
5. Before placing the containers into an insulated cooler (incubator) for 6-8 hours, use one cup to conduct a few simple observations. What is the consistency, pH (use litmus paper), color and smell. Be sure to make these same observations after the incubation period. During this "incubation time" the bacteria will multiply, ingest the milk sugar (lactose), and thicken the milk turning the mixture into yogurt. One word of caution, yogurt will not thicken or will separate if disturbed or bumped during the incubation period. Coagulation (thickening) changes the chemical makeup of protein so it is no longer water soluble (does not dissolve in water). Heat or acid at the proper temperature coagulates protein. In yogurt, protein is coagulated because acid is produced in a warm environment. If yogurt is moved during incubation (before yogurt is set), liquid and solid will separate.
6. After incubation, refrigerate, add fruit or other flavorings and enjoy!
7. Ask students to complete the "Microbes in My Food!" worksheet.

Activity 2: Sour Milk, Blue's the Clue

Prepare the lab by mixing 1 ml of methylene blue (1% solution) in 25 ml of water. Sterilize the test tubes, test-tube caps, pipettes (if using glass and not plastic sterilized pipettes). Purchase pasteurized whole milk and ultra high temperature (shelf stable) whole milk. (Shelf stable milk can usually be found in the juice aisle. Ask your store manager to order it if it isn't available in your supermarket.)

Place all the equipment on a lab table. Provide each student with "Blue's the Clue Data Table." Discuss pasteurization and UHT (shelf stable) milk with students. Ask students if

vocabulary:

- *microorganisims*
- *incubation*
- *fermentation*
- *pasturize*

notes:

they have ever wondered why their parents are always asking them to put the milk back in the refrigerator? What might happen to that milk if it's left out at room temperature overnight? What might be present in milk that if left out may cause the milk to spoil?

Design and Conduct Experiment

1. Ask students to form teams of 3 or 4 and encourage each team to develop a hypothesis on how temperature affects bacterial growth. Then ask them to design an experiment to test their hypothesis.
2. Introduce the three materials teams must use for their experiment: regular pasteurized milk, ultra high temperature (shelf stable) milk, and methylene blue.
3. Ask: How might you use methylene blue to help with your experiment? Students can research methylene blue and discover that it's an indicator dye used to determine the presence of bacteria in milk. Tell them they can use any of the other materials on the lab table. Also, there's a refrigerator and freezer they can use.
4. Explain that one container of milk came from the refrigerated dairy case of the supermarket and the other from an un-refrigerated shelf. Let students examine each container.
5. Let teams discuss their hypotheses and experimental designs for 10 to 15 minutes. Then, begin posing the following questions to help students design well thought out experiments:

   - What are some ways you could test the effect of temperature on bacteria? (Heating is a way to kill bacteria, whereas chilling and freezing are ways to retard the growth of bacteria.)
   - What's an important difference between the two milk products? Is there any information on the labels that relates to our question about the effect of temperature on bacterial growth? (Students should discover that one is pasteurized and the other is treated using ultra high temperature.)
   - What are the similarities and differences between pasteurized and ultra high temperature treatments? (Both pasteurization and ultra high temperature use heat to kill bacteria. Ultra high temperature methods use higher temperatures than regular pasteurization. Also, products treated at ultra high temperatures are packaged in special airtight containers to prevent bacteria from getting into the product.)
   - Could there be differences in the growth of bacteria between the two milks? What do you think the differences might be? (The regular pasteurized milk should show bacterial growth sooner than the UHT milk because the

pasteurized milk has more bacteria in it.)

- Should you consider these differences when you design your experiments? Why? (Yes, both milks should be tested in all conditions.)
- How can you tell if bacteria are growing in the test samples? (Add methylene blue to each sample. If bacteria are growing, the methylene blue will become colorless and the milk will change from blue to white. This is not immediate, but happens over a few days.)

6. Have each group present their hypothesis and experimental design to the class. Encourage students to discuss the merits of each suggested test. (One effective experimental design is to test pasteurized milk and UHT milk at three temperatures: room temperature, chilled, and frozen.)
7. After the group discussions, give the teams time to revise their hypotheses and experimental designs.
8. Let teams conduct experiments according to their designs. Note: The test tubes must be checked each day after the experiment is conducted. Since the color change happens over time, you could miss important findings if you don't check every day.

Observe and Record:

Students should make daily observations and record their results on the Blue's the Clue Data Table. Students should observe and record the time and any visual changes on day two of this lab activity.

Ask students:

- Did the data support or reject your hypothesis?
- What might happen if the chilled and frozen samples were left out at room temperature for several hours or overnight?
- Should we test them to find out? (Yes, let the chilled and frozen samples stand at room temperature until the following day. As they reach room temperature and remain in the Danger Zone for several hours, the bacteria will begin to grow. As this happens, the methylene blue will become colorless and the milk will change from blue to white. Observe and record the results.)
- What might happen if the UHT samples were left out at room temperature for another day? (If you let the UHT samples sit out at room temperature for another day or more, the color will change to white. Observe and record the results.)

notes:

notes:

Observe, Record, and Report:

Observe and record findings on the third day.

Ask students:

1. What happened to the frozen and chilled samples?
2. What happened to the UHT samples?
3. Give students 5 to 10 minutes to complete their Data Table.
4. Have teams present their findings to the class. They should report their results and discuss ways they would improve their experimental design.
5. Remind students to include the relationship of their findings to food safety.

Summary:

Temperature affects the growth of bacteria. Heating kills bacteria and chilling or freezing retards the growth of bacteria. Pasteurization is the process of destroying harmful bacteria that could cause disease by applying heat to a food; however, some spoilage bacteria may still be present. Bacteria grow more quickly in regular pasteurized milk than in UHT milk because the latter uses higher temperatures, thus killing more bacteria. Also, UHT milk is sealed in sterile, airtight containers. Results you can expect from this experiment:

Room temperature samples

- The pasteurized milk will turn white on the second day indicating that there are some spoilage bacteria in milk. At a temperature conducive to bacterial growth, they will multiply.
- The UHT milk will still be blue by the second day. This is because the UHT milk has fewer spoilage bacteria than regular pasteurized milk. Thus, it takes longer to see any bacterial growth. Bacteria do not quickly multiply in the UHT milk.
- After leaving the UHT milk at room temperature for another day or two, the color will turn white, indicating that spoilage bacteria will ultimately grow in the UHT milk.

Chilled and frozen samples

- Both the pasteurized and UHT chilled and frozen milk samples will still be blue by the second day, indicating that cold temperatures retard bacterial growth.
- After leaving the chilled and frozen samples at room temperature for another day or two, the color will change to white. This indicates that when the temperature rises to room temperature bacteria can grow. It may take longer for

the UHT milk to change to white because there are fewer spoilage bacteria in UHT milk than in regular pasteurized milk.

Extensions, Adaptations, Integration:

Activity 1a: Not-so Fool-Proof Yogurt

Try some real world science. Allow your students to manipulate the recipe on page 3 to see how bacteria thrive best. See numbers 6 and 7 on the Microbe worksheet for more experimental ideas.

Activity 2a: Sour Milk, Blue's the Clue

1. Compare the spoilage rate and bacterial growth in milk samples of varying fat content, such as powdered, skim, 1%, 2%, whipping cream, and half-and-half.
2. Study and discuss the numerous contributions of Louis Pasteur. (See references.)
3. Test UHT milk that has an expiration date that has passed and UHT milk that has an expiration date in the future. See if the "expired" milk changes more quickly than the fresher milk.

Activity 3: One Bad Apple Spoils the Bunch

How did the saying "one bad apple spoils the bunch" come about? To find out take one bad (rotting) apple and one crisp fresh apple and conduct the following test: Dip one sewing needle into rubbing alcohol to sterilize it. Pierce the rotten apple with the sterile needle. Immediately pierce the good apple. Tie a string around the stem of the good apple so you can identify it later. As a control, sterilize a second needle in the same way as the first. Pierce another good apple. (This apple will serve as a control in this experiment to insure that sticking an apple with a sterile needle will have no effect on the results.) Place the two good apples in a warm place and check them daily. Ask students to predict what will happen. (The apple with the string should develop a rotten spot; nothing should happen to the control apple.) The experiment demonstrates how sick people can spread germs by coughing or touching one another. If your friend has the flu, and coughs near you, it's possible that the cough can pass the microbes (viruses or bacteria) to you and make you sick.

Activity 4: Make cheese

Successful at making yogurt? You may want to try making cheese. It takes a bit more time (especially if you add a bacterial culture), but your students will be able to further investigate microorganisms and their use in food, the time it takes for bacteria to make cheese, and what bacteria need to thrive and reproduce. (See the Resource section for web sites that will help you with cheese making.) Don't want to take the time to make cheese but want to experience how bacteria changes milk into cheese?

notes:

notes:

"Microbes in My Food!"

Answer Key

1. fermentation
2. bacteria
3. the sugars have been converted to an acid
4. liquid to solid as a result of fermentation, a chemical change
5. to get the bacteria to multiply more quickly
6. too hot, it would kill the bacteria, too cold and the bacteria would not become active, consume the milk sugar and ferment the milk
7. nothing, no bacteria, no yogurt
8. the acid condition of yogurt (lack of sugar) keeps other bacteria from a food source
9. food, water, proper temperature, proper acid level (pH), no inhibitors present, host.

Have a cheese tasting party. Bring in or assign students to bring in different cheeses for students to sample. Create a chart that asks students to note if the cheese is sharp, mild, salty, squeaky, firm, etc. Take a class survey concerning the students' favorite cheese. Ask students to display the results graphically. Another strategy would be to apply the same experimental design used in Activity 2 to the yogurt experiment.

What are six things bacteria need to survive? Food, water, proper temperature, proper acid (pH), no inhibitors present, host.

How does/do _____ affect the growth of bacteria?

light
infrared light
ultra violet light
air/oxygen/carbon dioxide
soap (regular vs. antibacterial)
household disinfectants
temperature
moisture
antibiotics
salt
vinegar
sugar

Resources:

Methylene blue 1% (Educational Reagent Aqueous Solution available at most science supply stores or catalogs)

The Importance of Louis Pasteur
Author: Lisa Yount. 1994
Publisher: Lucent Books, P.O. Box 289011,
San Diego, CA 92198

Science and our Food Supply
Order free copy from www.foodsafety.gov/`fsg/teach.html

Yogurt web sites
www.dannon.com
www.yogurt.org.

Cheese making web sites
www.cheesemaking.com
www.agclassroom.org/ut

Materials adapted from Utah State University Extension and Utah Agriculture in the Classroom

Name_____

There Are Microbes in My Food!

1. What process caused a change in the original milk that you began with?

2. Why did you add a small amount of store-bought yogurt to your milk? What did this addition introduce into your milk mixture?

3. Why does the yogurt taste different from the original milk?

4. What changes occurred as the milk changed to yogurt? What caused these changes?

5. Why did the yogurt mixture need to be incubated in a warm container?

6. What if the milk mixture had been too hot or too cold, would the yogurt have set?

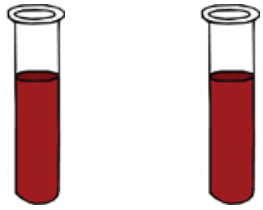
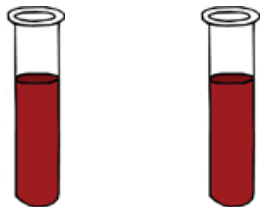
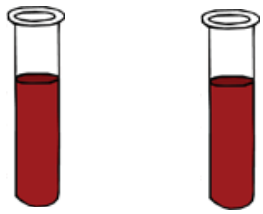
7. What would happen if the yogurt culture did not contain active cultures?

8. Why does yogurt keep better than fresh milk?

9. What are six things bacteria needs to survive?

Blue's the Clue

Name_____

Day 1 Original Sample	Day 2 Describe Visual Changes	Day 3 Describe Visual Changes	Day 4 Describe Visual Changes
Room Temperature  Pasteurized Milk UHT Milk	Pasteurized UHT:	Pasteurized: UHT:	Pasteurized: UHT:
Refrigerated  Pasteurized Milk UHT Milk	Pasteurized: UHT:	Pasteurized: UHT:	Pasteurized UHT:
Frozen  Pasteurized Milk UHT Milk	Pasteurized: UHT:	Pasteurized UHT:	Pasteurized: UHT:

1. How did the data support or reject your hypothesis?

2. What do you predict will happen if the chilled and frozen samples are left out at room temperature for another day?

3. What do you predict will happen if the UHT samples are left at room temperature for another day?

4. Explain the relationship of your findings to food safety.