“Bacteria in milk (not just an-udder microbiology lab)!”

Science in the Real World
Microbes In Action

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TABLE OF CONTENTS

BACTERIA IN MILK - NOT JUST AN-UDDER MICROBIOLOGY LAB

At a Glance

Description:

This is a two- day laboratory exercise in which the students will demonstrate the presence of bacteria in samples of milk. The samples differ in how they were pasteurized and the temperatures at which they have been stored and the date that they were purchased. On the first day, students will spread different milk samples on petri dishes containing nutrient agar. On the second day, colonies of bacteria will be counted and the class data averaged and analyzed. Concepts addressed in this lab include the presence of bacteria in fresh foods, the effect of temperature on bacterial growth and survival, the differences between pasteurization and sterilization, and the use of sterile technique in working with bacteria.

Time Requirements:

This exercise takes place over two days, but does not require a full period on either day. There is time for discussion and/or student working on questions and analysis on each day.

Curriculum Placement:

This exercise can be used in any life science course in conjunction with the following topics:

- introduction to bacteria
- microorganisms and food
- scientific method
- health and nutrition

Equipment:

To prepare materials and execute this lab you will need

- an autoclave or pressure cooker
- incubator at 32 degrees
- hot plate to boil water

Materials:

30 petri dishes (sterile, plastic)
nutrient agar to make 30 petri dishes
30 sterile applicators (swabs)
7 or 8 marking pens (1 per group)
4 test tubes (<130 mm long)
4 sterile transfer pipettes
2 half pint cartons of pasteurized milk
2 half pint cartons of UHT pasteurized milk
1 bio-hazard autoclave bag (optional

Time Line

Prior to the lab:

- teacher sterilizes tubes, makes nutrient agar and pours petri dishes
- teacher buys milk at least one or more weeks prior to the lab. This becomes the "old milk".

One week before lab:

- Teacher sets milk containers out at room temperature (see Preparations II)

One day before the lab:

- teacher buys 2 fresh containers of milk
- teacher sets one milk container out at room temperature

The day of the lab:

- Teacher boils part of the old milk sample for 5 min.
- Teacher boils part of the fresh milk sample, which was left out overnight, for 5 min.
- Students execute part 1 of the lab

Preparation

I. Materials

Per Class:
4 sterile tubes 16 x 125 mm (covered)
one half pint pasteurized milk as fresh as possible
one half pint UHT milk
2 sterile 5 ml pipettes
(sterile dropping pipettes can be substituted)
4 sterile dropping pipettes

For each group of 4 students

4 petri dishes containing nutrient agar
1 marking pen to mark on petri dish
4 sterile applicators (swabs)

II. Preparing Milk

At least one week before the lab get a half pint of milk from the cafeteria or grocery store. Also get at least two half pints of UHT pasteurized milk. One brand name of UHT milk is **Parmalat.** At the beginning of each class, point out the containers to these students and tell them these will be used in a lab activity next week. Leave these containers of milk out at room temperature for the week. These cartons of milk are to demonstrate graphically to the students the fact that pasteurized milk spoils when left at room temperature, while UHT pasteurized milk does not. The students will use other cartons of milk for their experiments, and the pasteurized milk they use will have to be purchased only a day or so before the lab begins.

III. Preparing Nutrient Agar

When convenient, but at least one day before the lab, nutrient agar must be prepared, sterilized and the agar poured into petri dishes. In addition, test tubes (at least 4 for each class) need to be sterilized. It is easier to use culture tubes, 16 x 125 mm, and caps to hold the milk samples. Regular test tubes covered with aluminum foil will also work. Note: The tubes should be short enough for the sterile droppers to stick out of the top when they are used.

The recipe for most nutrient agar is to dissolve 23 grams of nutrient agar powder into 1 liter of distilled water (tap water will work). Generally, figure on pouring 20-25 ml of nutrient agar into each dish. Prepare the agar in batches that only fill the flask half way to prevent boilover. Once you have prepared the nutrient agar, cover the flasks with aluminum foil and place them in the autoclave/pressure cooker. Also, be sure to put your sample tubes in the sterilizer at the same time (caps should be on loosely).
Follow the instructions on your sterilizer to complete the sterilization process. Generally, you need 15 lbs. of pressure and a temperature of 121º C for 15 minutes to achieve sterility.

Once the materials have been sterilized, the nutrient agar must be poured into the dishes before it solidifies. It is best to pour the agar when it has cooled enough to be held comfortably in your hand (45-50º C). Spread the plates out on the lab tables, lift the lid straight up and pour the agar into the dish. You may want to pour 20 ml of water into an empty dish to give you an idea of how much agar to pour into each dish. Once all of the dishes have been poured, let them set until they have solidified. Store the plates upside down until you are going to use them. If it is going to be several days until you use the plates, put them back into the plastic sleeve in which they where shipped and store them upside down in a refrigerator or a cool place.

IV. Sample Preparation

The day before the lab, you need to place the samples of milk (one sample from the UHT milk and three samples from a fresh carton of pasteurized milk) into the sterile tubes. You want to work as carefully as possible to maintain sterility. For the best lab results, you should use milk that is as fresh as possible, that is, with as late an expiration date as possible. Open the carton of milk as carefully as possible. Try not to touch the opening with your fingers. Using a sterile 5 ml pipette or a sterile dropping pipette, transfer about 5 ml of the pasteurized milk into each of 3 sterile tubes (if you have 5 classes that means a total of 15 tubes). Carefully, open the UHT milk and using a new sterile 5 ml pipette, transfer 5 ml of the UHT milk into a sterile tube (again, for 5 classes you will need 5 tubes) KEEP THE TUBES COVERED AT ALL TIMES WHEN NOT MAKING A TRANSFER. For each class, place one tube of fresh pasteurized milk into the refrigerator and leave two tubes at room temperature. The tube with UHT milk will also remain at room temperature.

V. Day One

On the day of the lab, be sure to have the petri dishes, cotton swabs and marking pens available to the students. Have the 4 stations set up, each with a tube containing a different sample of milk and a dropper. Label the tubes A, B, C, and D (do not make these letters correspond to the samples 1-4 that are described on the student pages (page 15). To begin class, show the class how you boiled one of the samples of pasteurized milk that had been left overnight at room temperature (put the tube into a boiling water bath for five minutes). While this is going on, move on to the containers of milk that were left out at room temperature for the past week. Open each container and ask the students what they expect the milk to look like, or smell like. Ask why this might have happened. Simply elicit answers from the students at this point, do not give too many details about bacteria and pasteurization. You can then pour out some of the milk from each container for the class to see. (If you have several classes, pour out only a small amount from each container, so that you will have enough for each class to see what has happened to the milk during the week.)

When the discussion has reached an end point, remove the tube that was in the boiling water bath and tell the students that one of the samples was treated this way. (You can use this tube as one of the four samples for the students, but they will then know which sample was the boiled one. It is perhaps better to have previously boiled a sample for the students to use and simply use the tube boiled in class to show how one of their tubes was handled.) Demonstrate to the students how to obtain their 2 drop sample of milk without contaminating the droppers or their petri dishes and how to use a sterile cotton swab to spread the milk over the surface of the agar. Emphasize the need to work quickly and carefully to help maintain sterility.

Divide the class into groups of four students each. At this point they can follow the instructions on the students pages.

1. Within the groups, have the students decide which sample each of them wants to test.

2. Have the students get their petri dishes and cotton swabs. The students will write their name, hour and sample letter on the bottom of their petri dish.

3. To avoid congestion in the lab, tell the students not to go to the station that has their sample if someone else is there. You may want to assign them the order in which to go to their station.

4. At their station, all the student needs to do is put 2 drops of the milk on the surface of the agar near the center and place the dropper back into the sample tube (they should not allow this dropper to touch anything besides the milk).

5. The student then takes the dish back to their lab area and uses the sterile swab to spread the milk over the surface of the agar. Have them place the swab back into

the paper it came in before they throw it away. Remind the students to check that their dish is labeled.

6. Collect the dishes and place them in an incubator at 32° C.

V. Day Two

On the following day, the students should observe their dish and the dishes of the others In their group. Review with them what a colony is and what they may look like. Have them fill out the data table from their group (Table A) and also add their data to the class data table on the board. Be sure they copy the class data onto the class data table (Table B) on their worksheet. The petri dishes must be collected from the students for proper disposal. They should either be autoclaved in a disposable autoclave bag (see supplies list) or they can be opened and flooded with 10% bleach for about 30 minutes before they are disposed of in the regular trash in a plastic garbage bag. Give the students time to analyze their data within their groups and to determine how their sample was handled. Follow this with a class discussion and tell the students how each sample was treated. If time permits, let the students begin to answer the questions on the worksheet.

Sterile Test Tubes

6 test tubes need to be sterilized for this activity. These may be capped and autoclaved along with the nutrient agar. If caps are not available, a square of aluminum foil will work.

Sources of Supplies:

Sigma Chemical Co.
P.O. Box 14508
St. Louis, MO 63178
(800) 521-0851

Description	Stock Number	Quantity	Cost
Nutrient Agar	N 0394	250 grams	$ 29.00
		500 grams	$ 59.00
Transfer Pipettes	Z 35,059	pkg. of 500	$ 41.00

Carolina Biological
2700 York Rd.
Burlington, NC 27215
(800) 334-5551

Sterile Applicators	F6-70-3033	box of 200	$ 16.00
Petri Dishes	F6-74-1350	case of 500	$ 90.00

TEACHER BACKGROUND

Bacteria are contaminants of all fresh foods. In order to avoid excessive spoilage, various measures can be employed to kill bacteria or to retard bacterial growth. These include keeping foods cold (or frozen), boiling (as is done for canned foods), salting (pickling), dehydrating (as in beef jerky), and adding anti-bacterial preservatives. In the particular case of milk, pasteurization combined with refrigeration is the most common technique used. Pasteurization does not kill all the bacteria (or spores) in milk, but does eliminate most of the pathogenic bacteria that have been historically associated with milk, such as tuberculosis, brucellosis, and typhoid. Pasteurization was first developed in order to kill these pathogens, but it was soon discovered that this process also improved the keeping quality of the milk without sacrificing the taste. Pasteurization can be accomplished by heating milk to 63-650 C for 30 minutes or to 71° c for 15 seconds (flash pasteurization) followed by rapid cooling. Flash pasteurization is the most common technique used. Pasteurization does not prevent spoilage, but it reduces the bacterial population so that spoilage occurs more slowly. Milk can be essentially sterilized by Ultra High Temperature (UHT) pasteurization in which it is heated to a higher temperature than is used for normal pasteurization, but just for a few seconds (149° C for 6 to 9 seconds). This milk can be stored for several months at room temperature without spoiling.

Usually an investigation of the bacterial content of milk involves doing serial dilutions of the milk, since the bacteria can easily number in the tens of thousands per milliliter without the milk being "spoiled". Milk cannot be sold if it contains over 30,000 bacteria per mL, but it usually contains only a small number (hundreds or less) when it is newly pasteurized. In order to accommodate this investigation to the high school classroom, we have avoided using serial dilutions. By using milk that is very fresh (well before its expiration date) and only plating 0.1 ml (two drops) of the refrigerated milk the numbers of the bacteria in the refrigerated, pasteurized milk sample is usually in a good range for counting colonies. The sample that has been left at room temperature will have significantly more colonies (often too many to count). Boiling the room temperature sample usually kills all the bacteria and plates using this sample should not have any colonies. The same is true for the UHT pasteurized milk sample.

There are a variety of bacteria that can be present in milk. Among other differences, they vary in the temperatures at which they will grow optimally. Some are described as psychrophilic, which means that they grow best at cold temperatures, while others are severely retarded by being in the refrigerator and grow rapidly only at warmer temperatures. This lab does not distinguish among the various kinds of bacteria. Often there is more than one kind of colony visible on the petri dishes, and the fact that there are several bacterial species present may be a point to discuss with the class.

Teacher Hints and Troubleshooting

1. The fresh milk used in this experiment must be really fresh. Milk approaching the "Buy by..." date is too old.

2. The old milk and the "left out" sample may have too many bacteria to count. They will produce a "lawn" of bacteria, the term scientists use to describe continuous growth.

3. Incubation temperature for the dishes is important. Room temperature is too cool to react overnight. 32 degrees is the ideal temperature for growth.

4. Dishes soaked in bleach can be disposed of in regular trash. Drain the bleach down the drain first to avoid a mess.

ANSWER KEY

1. How did the number of bacteria in the pasteurized milk from the refrigerator compare with the number in the pasteurized milk that had been kept at room temperature for 24 hours?

   Answers will vary, but students should see significantly more growth (a greater number of colonies) in the room temperature sample.

   Why do you suppose they are different?

   Most bacteria reproduce faster at room temperature than at 4° C. Refrigeration retards bacterial growth.

2. a. Two drops is equal to about 0.1 ml. How many bacteria were present in two drops of the pasteurized milk that had been kept in the refrigerator?

   Answers will vary, but students should see significantly more growth (a greater number of colonies) in the room temperature sample.

   b. How many bacteria would be present in each milliliter of the sample?

   10 times the above.

   c. A half pint contains 236 ml. How many bacteria were present in the entire half Pint of milk?

236 times the previous number.

3. Why can the ultra high temperature (UHT) pasteurized milk be stored at room temperature for months before opening, while regular pasteurized milk cannot? Use the results of the experiment to explain your answer.

   UHT milk is essentially sterile—that is, all the microorganisms in the sample have been killed. As long as it is packaged sterilely and is not opened, nothing should grow in it. Pasteurized milk has living bacteria (or bacterial spores) in it—they are not all killed. This milk will thus spoil as these bacteria grow

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4. How did the number of bacteria in the pasteurized milk that had been left at room temperature compare with the same milk that had been boiled for five minutes?

   The boiled milk had far fewer (or no) colonies.

   What does boiling do to the bacteria?

   Boiling kills most bacteria.

5. Your new job is in the Health Department's Food Safety Division. You have been assigned the task of comparing the number of bacteria in two different samples of hamburger. One is regular hamburger and the other has had a new preservative added that is supposed to slow down the growth of bacteria in hamburger. Your supervisor has asked you to give her an outline of how you plan to proceed with this assignment. Use the space below for your outline.

   Answers should include some method for sterilely getting a small, measured sample of hamburger spread on the agar dishes (it could be rubbed with a swab or it could be ground in water and then some of the liquid spread on the agar). The dishes should be incubated at a warm temperature for a day or so and then colonies should be counted to determine the number of bacteria in the sample.

Name_____

Date_____

BACTERIA IN MILK- NOT JUST AN-UDDER MICROBIOLOGY LAB

BACKGROUND

Bacteria are one of the biggest competitors for our food supply. We are constantly struggling to prevent food from spoiling. Spoilage is the result of the action of bacteria and other microorganisms.

In this lab exercise you are going to determine if bacteria are present in milk. Milk can have bacteria in it, either from the cow (if it is ill) or from the handling of the milk before it is packaged and delivered to the store. The milk most of us buy has been pasteurized to help prevent spoilage due to bacteria. During pasteurization, the milk is usually heated to 71° C for 15 seconds. Another technique for helping to preserve milk is called ultra high temperature (UHT) pasteurization, during which milk is heated to 149° C for 6 to 9 seconds. One thing you will do in this exercise is to compare milk that has been pasteurized by these two methods.

Given the extremely small size of bacterial cells (1-3 micrometers in diameter), special techniques must be used in order to be able to actually "see" the bacteria. One way to do this is to spread the material you think might contain bacteria onto the surface of a special material called nutrient agar. Nutrient agar is a jello-like material that contains all of the nutrients bacteria need to grow and multiply like crazy. In this exercise, the nutrient agar has been poured into a shallow, round dish (a petri dish). When the bacteria are spread onto the agar they begin to grow and divide rapidly. How rapidly they divide will depend on many factors, such as the presence of adequate food and moisture. Many bacteria grow best around 37° C. Others require warmer or cooler temperatures. Under the best conditions, bacteria may divide as fast as once every twenty minutes! Since the bacteria cannot move over the agar surface, each cell will give rise to a mass of bacteria (millions of cells) called a colony that can be seen by the naked eye. Each colony on the plate originates from a single cell. By counting the colonies on the plate you can determine how many bacteria were in the original sample.

At this point, it is important to note that you only want to grow bacteria that came from the material you are testing, so you must be careful not to introduce bacteria from other sources—the air or you, for example. The equipment you will use has been sterilized, so there are no bacteria present on this equipment. When working, you must work as carefully as possible to prevent contamination of the petri dish. Always leave the lid on the dish until you need to put something on the surface of the agar. When you need to open the dish, lift the lid straight up and hold it over the petri dish, place the material on the surface of the agar and immediately put the lid back on. Each time it is necessary to open the petri dish, follow these precautions.

PURPOSE- DAY ONE:

1) To determine if bacteria are present in different samples of milk.
2) To determine the effect of temperature on the number of bacteria present in a sample of milk.

MATERIALS PER GROUP OF 4 STUDENTS:

4 petri dishes with nutrient agar
4 sterile applicators (cotton swabs)
1 marking pen
Access to tubes of milk samples with sterile droppers

DAY ONE PROCEDURE:

1. Work in groups of 4. Each student in the group should pick one letter (A, B, C, or D). This will be the sample of milk with which you will work.

2. Each student in your group needs one petri dish. Note: the contents of the petri dish are sterile—do NOT open the dish. On the bottom of the petri dish (the part that contains the nutrient agar) write your name, hour, date, and the letter representing your milk sample. Write in small letters near the edge of the dish bottom.

3. The teacher has placed the milk samples at different locations in the room. Find the sample you are to test and take your petri dish to the appropriate station.

4. Once at your station you are ready to place the sample of milk on the surface of the agar. You must work quickly and carefully so that you don't introduce any bacteria from the environment into the petri dish. Remember that when you open your petri dish, lift the lid straight up and hold it over the dish for as short a time as possible to carry out the operations you need to perform. When you are ready, use the dropper (it is sterile—don't let the tip touch anything) to put two drops on the surface of the nutrient agar near the center of the dish. Place the dropper back in its tube.

5. Carefully carry your dish back to your lab station. You will now need a cotton swab. This swab is sterilely wrapped—do not unwrap it until you are ready to use it. When you are ready, unwrap the swab (do not touch the cotton end), spread the milk over the surface of the agar as evenly as possible. When finished, place the swab back into the paper wrapping and dispose of it in the garbage.

6. When all four students in your group are finished, stack the dishes and tape them together. Your teacher will give you instructions on where to put these.

The four milk samples that your group worked with were:

1. pasteurized milk that has been kept in the refrigerator (about 4° C)

2. the same pasteurized milk that has been kept at room temperature (about 22° C) for 24 hours.

3. pasteurized milk that was kept at room temperature for 24 hours and then was put in a boiling water bath for 5 minutes.

4. ultra high temperature (UHT) pasteurized milk that has been kept at room temperature for 24 hours

(Note: the order above does not correspond to samples A through D)

On your Results and Analysis sheet, answer questions 1 and 2.

DAY TWO

DAY TWO PROCEDURE:

1. Your teacher will return your petri dishes to your group. Untape them and get the dish you made.

2. Look for colonies on the agar in your dish. Remember, a colony is a visible mass of bacteria that is produced from one original bacterial cell that ended up in that position after you spread the sample on your petri dish. Count the number of colonies on your dish and record this number in Table A. Be sure to look at the dishes of the other members of your group and record their results in Table A.

   (**Note**: Sometimes there are so many bacteria on your plate that they completely cover the surface of the agar. This is called a lawn of bacteria. At other times, there may be individual colonies, but there are too many to count (TMTC). Use these terms in Table A if needed.)

3. Each individual needs to place his/her results on the data table for the class (provided by the teacher). When all the data are entered, record the class data in Table B.

4. Using the class data, try to determine which type of milk corresponds to each of the samples (A, B, C, D). Be prepared to explain your answers.

5. Follow your teacher's instructions for disposing of the petri dishes. They having living bacteria on them and cannot be disposed of without special treatment.

.

Name _____
Date _____

RESULTS AND ANALYSIS

1. Using the information at the end of the Day One Procedure about milk samples 1 -4, predict which sample will have the greatest number of living bacteria in it and explain why you think so.

2. Which samples do you think will have the least number of bacteria"? Explain your choice.

Results:

Table A (your group's results)

Sample	Number of Colonies
A	
B	
C	
D	

Table B (class results)

Sample	Number of Colonies from Different groups	Average Number of Coloines
A		
B		
C		
D		

Analysis

1. How did the number of bacteria in the pasteurized milk from the refrigerator compare with the number in the pasteurized milk that had been kept at room temperature for 24 hours?

   Why do you suppose they are different?

2. a. Two drops is equal to about 0.1 ml. How many bacteria were present in two drops of the pasteurized milk that had been kept in the refrigerator?

   b. How many bacteria would be present in each milliliter of the sample?

   c. A half-pint contains 236 ml. How many bacteria were present in the entire half-pint of milk?

3. Why can the ultra high temperature (UHT) pasteurized milk be stored at room temperature for months before opening, while regular pasteurized milk cannot? Use the results of the experiment to explain your answer.

4. How did the number of bacteria in the pasteurized milk that had been left at room temperature compare with the same milk that had been boiled for five minutes? What does boiling do to the bacteria?

5. Your new job is in the Health Department's Food Safety Division. You have been assigned the task of comparing the number of bacteria in two different samples of hamburger. One is regular hamburger and the other has had a new preservative added that is supposed to slow down the growth of bacteria in hamburger. Your supervisor has asked you to give her an outline of how you plan to proceed with this assignment.
   Use the space below for your outline.