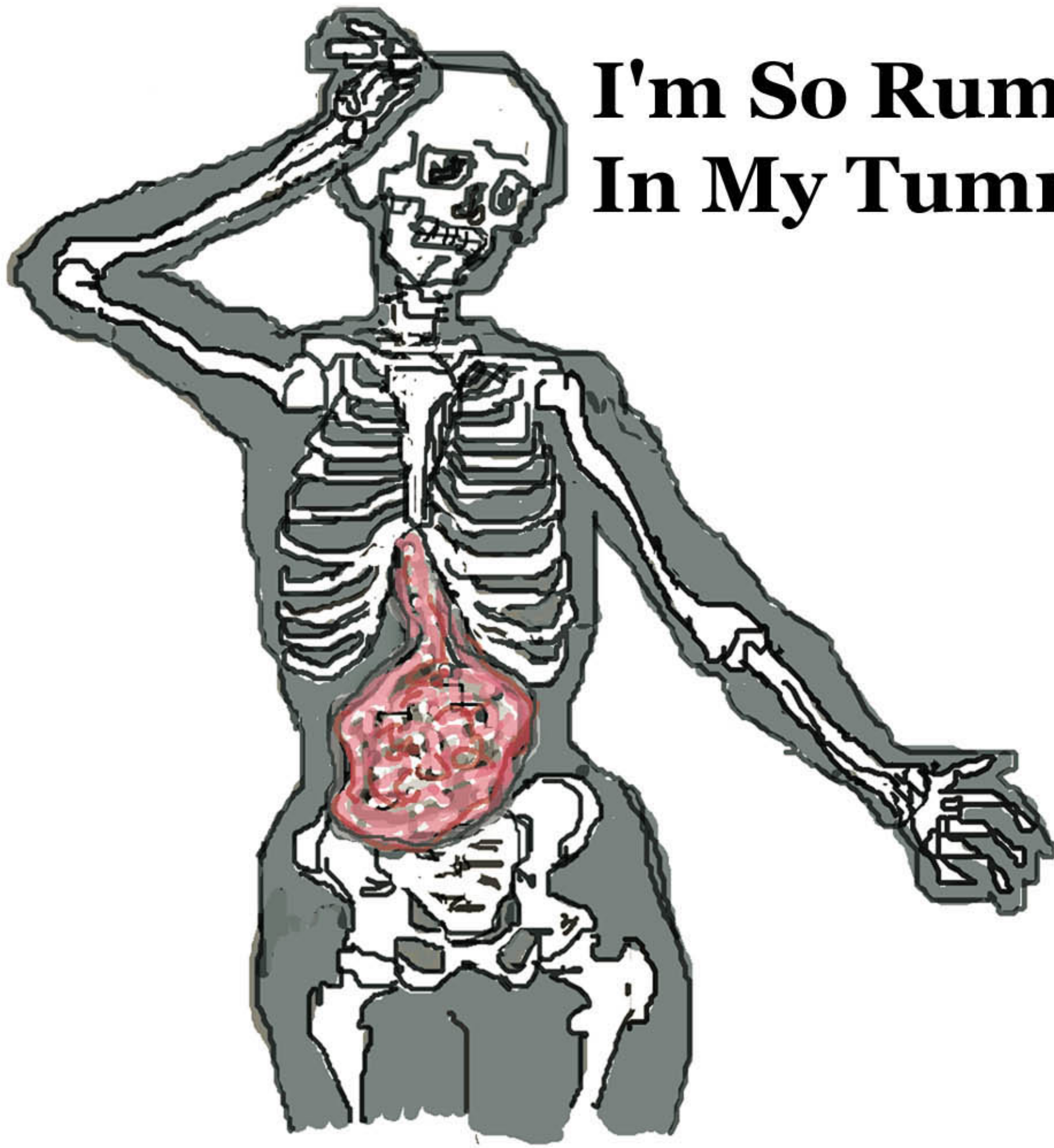
SCIENCE IN THE REAL WORLD

Microbes in Action



I'm So Rumbly In My Tummy. . .

A Laboratory Exercise on Lactose Intolerance

Science in the Real World
Microbes In Action

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At a Glance

Description
This lesson consists of two laboratory exercises. In the first lab, Tiny Bubbles, students observe the products of the fermentation of the sugar lactose by *E. coli.* In the second lab, Tickled Pink, they will simulate in test tubes what happens in the intestines of lactose intolerant and lactose tolerant individuals when they drink milk products.

Time Requirements
This unit will require part of five laboratory periods.

Curriculum Placement
This activity could be used to teach the concepts of fermentation, enzyme activity, digestion, or as part of a microbiology unit.

Equipment
test tube racks
marking pens
inoculating loops
Bunsen burners
incubator
sterilizer/autoclave
matches or flint striker

Timeline
Order *E. coli* culture to arrive one or two weeks before the lab. You must use an intestinal strain. Ordinary lab strains of *E. coli* will not work.

Prepare fermentation tubes and sterile water 24 hours before the lab. This can be done several weeks in advance of the lab.

One day before the lab: Grow *E. coli* culture in nutrient broth.

Day 1: Students inoculate fermentation tubes with *E. coli.*

Day 2: Students observe test tubes and answer questions.

Day 3: Instructor prepares *Lactobacillus* suspension. Instructor prepares *E. coli* broth culture for day 4. Students inoculate litmus milk with *Lactobacillus* suspension.

Day 4: Students inoculate test tubes with *E. coli* broth and incubate for 24 hours.

Day 5: Students observe incubated test tubes and analyze the data.

Tiny Bubbles: Lactose Fermentation by *E. coli*

Background
Microbes are present everywhere in our world. Their metabolism and growth characteristics can vary with the environment in which they find themselves. Changing even one aspect of their environment may change the way they grow.

All living organisms require nutrients, particularly a good source of carbon. Sugars such as sucrose, glucose and lactose (the sugar found in milk) are good sources of carbon for animals and for most bacteria. Various organisms use sugars by different metabolic pathways, making a variety of products. As sugars are degraded during metabolism the carbon bonds are broken, often releasing carbon dioxide as a gas.

The *E. coli* used in this lab is part of the normal flora of the human intestine. *E. coli* metabolizes the sugar lactose, producing carbon dioxide gas. In this lab you will compare the metabolism of *E. coli* when it is grown in a nutrient medium with or without lactose.

Based on the information provided, answer the following questions and record your answers on the Student Data Sheet.

1. State the problem you are investigating.

2. Write a hypothesis using an if-then format.

3. What is the independent variable in this experiment? What is the dependent variable?

4. What conditions remain constant?

Materials (per team of two students)

1 tube of sterile nutrient broth with a gas collecting tube, labeled tube “A”
1 tube of sterile lactose broth with a gas collecting tube, labeled tube “B” (lactose broth is nutrient broth with lactose added)
1 fresh broth culture of *E. coli*
1 test tube rack
1 sterile transfer pipette marked at 0.5 ml
1 Bunsen burner
matches or flint striker
marker

Day One Procedure

Work in teams of two to carefully complete the transfers described below. Use sterile technique as instructed by your teacher.

1. Swirl the overnight *E. coli* culture to mix the contents.

2. Using a sterile transfer pipette, withdraw 0.5 ml from the overnight *E. coli* culture.

3. Carefully add 0.5 ml of the culture in the pipette into test tube “A” containing the sterile nutrient broth.

4. Using the same sterile transfer pipette, again withdraw 0.5 ml from the overnight *E. coli* culture.

5. Add 0.5 ml of the culture in the pipette into test tube “B” containing the sterile lactose broth (nutrient broth and lactose).

6. Dispose of the pipette in the disinfectant solution.

7. Place both test tubes “A” and “B” in an incubator at 37°C.

Day Two Procedure

At the end of 24 hours observe the test tubes for the presence of gas in the inverted gas collecting tubes. The carbon dioxide appears as a bubble inside the gas collecting tube. Record your observations on the Data Sheet.

Name ______________________________

Date________________

Tiny Bubbles Student Data Sheet

1. Problem ____

____

2. Hypothesis ____

____

3. Independent variable ____

   Dependent variable ____

4. Constant conditions ____

____

Observations

5. Describe the difference(s) observed between the two tubes. Sketch and label what you see.

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6. Write a short summary paragraph describing the difference in the two "test tube environments" and explain your results.

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Tickled Pink

Background
In the movie “French Kiss” with Meg Ryan and Kevin Kline, there is a scene where Meg Ryan becomes ill and yells “lactose intolerance” as she runs from the train to a rest room. Maybe you have seen TV commercials for products such as Lactaid which allow lactose intolerant individuals to eat foods such as ice cream or cheesecake. Do you know what this condition is? Do you know what it feels like? Why do some people suffer from this condition and others don’t? What is going on in your “tummy” when this happens, and what does this have to do with microbes and metabolism?

In this laboratory activity, you will simulate what goes on in the human intestines of individuals who are lactose tolerant compared to those who are lactose intolerant.

In the small intestine, lactose, a disaccharide found in milk, is broken down by the enzyme lactase into glucose and galactose, two simple sugars. This reaction is as shown below:

$$\underset{\text{lactose}}{C_{12}H_{22}O_{11}} + \underset{\text{water}}{H_2O} \rightarrow \underset{\text{glucose*}}{C_6H_{12}O_6} + \underset{\text{galactose*}}{C_6H_{12}O_6}$$

These simple sugars are quickly absorbed through the wall of the small intestine and distributed into the cells of the body to use as energy.

In a **lactose intolerant** individual, the lactose is not converted to glucose and galactose in the small intestine. Since lactose cannot be absorbed by the small intestine, it travels to the large intestines. It is then metabolized by bacteria, such as *E. coli,* that are normally found in the large intestine. In this laboratory, you will simulate this metabolism in a test tube.

In addition, you will simulate what occurs in the intestines of **lactose tolerant** individuals. You will use lactic acid bacteria, properly called *Lactobacillus* bacteria in this model. We use this bacterium because just as the human intestines make the enzyme lactase to break down lactose into simple sugars, so does this bacterium. The *Lactobacillus* bacteria, present in yogurt, further metabolize the glucose and galactose to lactic acid, which is excreted from the bacterial cell as waste. This is the chemical that gives yogurt its sour taste.

lactose ——> lactic acid

*Note that several 6-carbon sugars contain the same number of carbon, hydrogen and oxygen atoms, but they are arranged in different structures; hence, they are different compounds.

Materials (per team of two students)
1 broth culture of *Lactobacillus sp.* (from yogurt)
1 broth culture of *E. coli (needed only on Day 2)*
4 culture tubes of litmus milk
2 sterile transfer pipettes

Day One Procedure

Work in teams of two to carefully complete the transfers described below. Use sterile technique as instructed by your teacher.

1. Obtain 4 test tubes of litmus milk. Label the tubes “A,” “B,” “C” and “D.”

   A. No bacteria
   B. *Lactobacillus*
   C. Mixed culture of *Lactobacillus,* followed on Day 2 by *E. coli*
   D. *E. coli* (to be inoculated on Day 2)

2. Gently tap the side of the suspension of *Lactobacillus* to swirl the contents.

3. Using a sterile transfer pipette, withdraw 0.5 ml of *Lactobacillus* from the suspension and add the entire amount to test tube “B.”

4. Using the same sterile transfer pipette, again withdraw 0.5 ml of *Lactobacillus* from the suspension and add the entire amount to test tube “C.”

5. Dispose of the pipette as instructed by your teacher.

6. Observe the test tubes and record their appearance on the Data Sheet.

7. Incubate all test tubes at 37°C overnight.

Day Two Procedure

Obtain your tubes from day one. This includes the untreated tubes “A” and “D,” and the two tubes inoculated with *Lactobacillus.*

1. Do not shake the test tubes. Observe them and record your observations on the Data Sheet.

2. Put test tube “A” (no bacteria) aside and do NOT open it. Put the overnight culture marked “B” (*Lactobacillus*) aside and do NOT open it.

3. Swirl the *E. coli* culture to mix the contents.

4. Using a sterile transfer pipette withdraw 0.5 ml from the *E. coli* culture and add it to the test tube marked “D” (*E. Coli*).

5. Using the same sterile transfer pipette withdraw 0.5 ml from the *E. coli* culture and add it to the test tube marked “C” (mixed culture).

6. Incubate all test tubes at 37°C overnight.

Day Three Procedure

1. Obtain your tubes from day two. These are the 4 tubes which were treated as follows:

   A. No bacteria
   B. *Lactobacillus*
   C. Mixed culture of *Lactobacillus,* followed later by *E. coli*
   D. *E. coli*

Do not shake the test tubes. Observe them carefully. Record your observations on the Data Sheet.

Name ______________________________

Date ______________________________

Tickled Pink Student Data Sheet

Data and Analysis

Day One Observations				
	A. No bacteria	B. *Lactobacillus*	C. Mixed culture	D. *E. coli*
Gas present?				
Color?				
Texture?				

Day Two Observations				
	A. No bacteria	B. *Lactobacillus*	C. Mixed culture	D. *E. coli*
Gas present?				
Color?				
Texture?				

Day Three Observations				
	A. No bacteria	B. *Lactobacillus*	C. Mixed culture	D. *E. coli*
Gas present?				
Color?				
Texture?				

1. Did tube “A” (no bacteria) change from day two to day three? Explain.

____

____

2. Litmus milk contains the indicator litmus that changes color to show the presence of an acid or a base. Pink indicates the presence of an acid and blue shows a base. On the pH scale, an acid has a pH value of less than 7 and a base has a pH value of over 7. Purple is neutral, indicating a pH of 7. On the table below, indicate the acid/base condition of each tube.

Test tube	Color	Explanation
A		
B		
C		
D		

3. What can be determined about the pH from the color of the litmus milk in test tube “B” (*Lactobacillus*)?

____

____

4. How did the color of the litmus milk in test tube “C” (mixed culture) compare to the color in the other tubes? What does that indicate?

____

____

5. Summarize the data tables above comparing all tubes to each other. Be specific about results.

____

____

6. Does *Lactobacillus* produce gas when it ferments the lactose in milk?__________

7. What happens when *E. coli* ferments lactose?

____

____

____

8. What will happen if *E. coli,* while living in the large intestine, finds lactose in the environment?

____

____

9. Refer to tube “C” (mixed culture). If *Lactobacillus* is allowed to ferment milk **before** the milk is inoculated with *E. coli,* will *E. coli* be able to produce gas? Explain your answer.

____

____

Read the paragraph below and answer the questions that follow.

Lactose intolerance is a condition found in many people. It is the inability to break down milk sugar, called lactose, during digestion. Sometimes it is caused by a defective gene for the enzyme lactase. This enzyme breaks down lactose. Other times the condition develops over time and is evident in older adults. Because lactose is not broken down, it passes through the digestive tract and into the large intestine. The presence of lactose in the large intestine provides a food source for the intestinal bacteria that normally live symbiotically in the human large intestine. One of these intestinal bacteria is called *Escherichia coli (E. coli).* Intestinal bacteria get nutrients from the remains of the food that we have eaten. In return, they help us by producing vitamins, which we absorb and use. As they ferment lactose, carbon dioxide gas is produced leading to the gastrointestinal distress or cramping that is associated with lactose intolerance.

10. What part of the digestive process is not functioning properly in lactose intolerant individuals?

____

____

11. What substance in milk can be used by intestinal bacteria, as a food source during fermentation?

____

____

12. *E. coli* and other bacteria live in the large intestine of **all** individuals. Why then do most individuals not suffer when they drink milk?

____

____

13. What is the probable cause of the “rumbly tummy” that lactose intolerant individuals feel when they drink milk?

____

____

14. Lactose intolerant individuals can often eat cultured milk products such as yogurt, buttermilk, or acidophilus milk with no problems. What did the bacteria do to the milk that now allows these individuals to eat the product without having symptoms?

____

____

15. Design an experiment to test this question, “Is lactose broken down equally well by the bacteria found in different types of cultured milk products (i.e. yogurt, buttermilk, acidophilus milk)?” For this experiment, state the problem, form a hypothesis, list materials and protocols needed. If time permits, you may want to do this lab.

Teacher Guide

Background

Lactose Digestion in Humans

Milk and milk products are nutritious foods, containing the disaccharide lactose, the protein casein and fats in the form of cream. However, some people experience difficulty digesting the milk and are said to be lactose intolerant. Even people who drank milk or ate milk products without difficulty in their childhood can experience some degree of lactose intolerance as they age. For these people, the process of digesting the lactose into the resulting monosaccharides, glucose and galactose, does not occur as it should in the small intestine. This may be due to a defective gene for the enzyme lactase or insufficient amounts of the lactase enzyme that is produced by the cells lining the walls of the small intestine. Without the enzyme lactase in the small intestine to do its job, lactose passes undigested into the large intestine.

Many bacteria, including *E. coli,* are normal inhabitants of the human large intestine. There they have favorable growing conditions: continual food sources and a warm temperature (37°C). The large intestine is an anaerobic environment containing little or no oxygen. Because of the anaerobic conditions, intestinal bacteria must rely on fermentative metabolism. If sugars are present in the food source, bacteria can ferment them to various products, including carbon dioxide gas and various acids.

Carbon dioxide gas produces abdominal distress, resulting in painful or uncomfortable cramps. Normally, in the small intestine disaccharide sugars like lactose, sucrose, and maltose are digested to simple monosaccharides that are easily absorbed into the bloodstream. This process of the small intestine prevents sugars from reaching bacteria that inhabit the large intestine. Therefore, intestinal bacteria normally metabolize a sugar-free food source. Without sugar, intestinal bacteria cannot carry on the fermentation pathway that produces carbon dioxide.

Lactose intolerant people, who lack the enzyme lactase, can avoid the problems caused by lactose if they employ the services of microbes. The enzyme lactase is commercially produced from yeast and can be purchased in tablet form or already added to milk.

Lactose Fermentation in Bacteria

The lactic acid-producing bacteria ferment lactose anaerobically (in the absence of oxygen) to only one end product, lactic acid. They have been used for centuries all over the world to produce yogurt and cheeses. For example, yogurt is called leben in Egypt, matzoon in Armenia, naja in Bulgaria and dahi in India. Yogurt is made by adding *Lactococcus* (formerly called *Streptococcus*) *thermophilus* and *Lactobacillus bulgaricus* to milk.

There are also a variety of fermented milk beverages that have been made for centuries in various countries, especially in Eastern European countries. For specific flavors and acidity, specific organisms or groups of organisms are added to milk. Cultured buttermilk is made by adding *Lactococcus cremoris* to pasteurized skim milk. Other organisms, *Lactococcus lactis, Streptococcus diacetylactis* and *Leuconostoc* *citrovorum, Lactobacillus cremoris* or *Lactobacillus dextranicum* give buttermilk different flavors. Sour cream is made by adding one of these organisms to cream. Acidophilus milk is made by incubating milk inoculated with *Lactobacillus acidophilus*.

Other *Lactobacillus* products are economically important too. Cheeses are an important food group to many people. The first step in making almost any cheese is to prepare a curd by adding *Lactobacillus* and either rennin or bacterial enzymes to milk. The curds, which result from the precipitated proteins are then processed into cheese. Sourdough breads, gaining in popularity, are also an example of the use of lactic acid producing bacteria in food production.

The souring of milk is a good example of ecological succession. Raw milk, after it is collected, contains small numbers of many different bacteria. The original warm temperature of milk plus the neutral pH and presence of the milk sugar lactose favor the rapid multiplication of strains of *Lactococcus,* such as *Lactococcus lactis.* The lower pH resulting from the lactic acid they produce eventually inhibits the growth of *Lactococci.* They are succeeded by the *Lactobacilli* species, which tolerate the lower pH. They decrease the lactose concentration further and lower the pH even more. Then other microbes such as yeast can follow the *Lactobacilli.* Yeast can metabolize the lactic acid and tolerate the low pH. By using up certain nutrients, changing the pH, and producing metabolic wastes, each microbe helps create conditions favorable for another group.

pH Indicators

Litmus has been used as an indicator to detect acid since the late 1800s. Litmus is a substance extracted from certain lichens. Its exact chemical composition is not known, although it is known to consist of phenol containing compounds. Litmus is light blue or gray around pH 7; it is pink below pH 5 and is blue-violet above pH 8. Litmus is colorless when no oxygen is present in the solution. Litmus can become colorless when bacteria deplete the oxygen and grow anaerobically by fermentation. Litmus can also become colorless during autoclaving. When it becomes colorless, litmus can no longer indicate the pH of the medium. In the inverted gas collecting tube, the oxygen is driven out and the solution cannot re-absorb oxygen while the tube is inverted. So the solution inside the gas collecting tube remains colorless.

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. making inferences about a model system

2. demonstrate the following laboratory skills:

    a. using sterile technique
    b. using a sterile transfer pipette to measure volume
    c. constructing a data table
    d. comparing and contrasting

3. demonstrate the understanding of the following scientific concepts:

    a. fermentation by:
        I. explaining the color change seen in the reaction tubes
        II. identifying fermentation as a bacterial process

    b. microbial metabolism by:
        I. describing the use of lactose as a food source
        II. identifying the pH change as indicative of acid production during lactose fermentation
        III. stating the differences seen between *E. coli* metabolism of sugar, alone and in the presence of *Lactobacillus*

    c. pH indicators by:
        I. identifying a color change as indicative of a pH change
        II. explaining how a pH change can reflect chemical reactions

    d. lactose intolerance by:
        I. explaining the normal breakdown of lactose in human digestion
        II. identifying *E. coli* as one of the fermenting organisms that causes the symptoms of lactose intolerance

Sources of Supplies
Containers of yogurt labeled "active yogurt cultures" (Dannon plain yogurt works well) were purchased at the supermarket.

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

Description	Stock Number	Quantity
Lactose broth	K3-78-4060	100 g
Nutrient broth	K3-78-5360	100 g
Litmus milk	K3-70-4280	100 g
1 ml pipettes	K3-21-5830	250
Small culture tubes (6 x 50 mm)	K3-73-1402A	72
Culture tubes (18 x 150 mm)	K3-73-1464	case/500
Intestinal *Escherichia coli* culture	K3-15-5065	1 tube

Preparation

Tiny Bubbles Materials (per pair of students)
2 transfer pipettes
1 test tube of nutrient broth
1 test tube of nutrient broth plus lactose
E. coli culture
10% disinfectant solution
2 small culture tubes

Tickled Pink Materials (per pair of students)
4 culture tubes of litmus milk
Lactobacillus broth culture (from yogurt)
E. coli culture
10% disinfectant solution
4 small culture tubes

Media
For each student group, prepare:
4 tubes litmus milk with small gas collecting tube
1 tube of nutrient broth with small gas collecting tube
1 tube of lactose broth with small gas collecting tube
2 tubes of nutrient broth without gas collecting tube

Prepare the following:

1. **Litmus milk (1 liter)**
   100 grams of litmus milk
   1 liter deionized water

2. **Nutrient broth (500 ml)**
   4 grams nutrient broth powder
   500 ml deionized water

3. **Lactose broth (500 ml)**
   6.5 grams lactose broth powder
   500 ml deionized water

4. **100 ml water** (autoclave for *Lactobacillus* suspension)

Dispense 7 ml of medium (litmus milk, nutrient broth or lactose broth) into large culture tubes. For litmus milk, lactose broth and one-third of the nutrient broth tubes, drop the small gas collecting tube into the larger tube. The open end of the small tube goes in the larger tube first. The small tube will automatically fill during autoclaving. Place caps loosely on tubes.

Autoclave the tubes at 15 lb. pressure (121˚C) for 15 minutes (do not autoclave the litmus milk too long or the milk may curdle).

Once the gas collecting tubes have filled with the litmus milk they will remain pale yellow because of the anaerobic conditions. All color changes should be based on the solution in the surrounding larger test tube.

Cultures:

1. ***E. coli** culture (made in advance)**

   Using a sterile inoculating loop, transfer a small amount of *E. coli* from a slant or plate to tubes of nutrient broth (without a gas collecting tube) for each student group. Incubate at 37°C overnight.

2. ***Lactobacillus* suspension (100 ml)**
   10 ml plain yogurt with active cultures (Dannon always works well)
   100 ml sterile water

***This strain is not pathogenic. Pathogenic strains of *E. coli* such as the strain *E. coli* O157:H7 contain additional genetic information that allows them to produce toxins. The normal *E. coli* in the human intestines lacks these genes and, hence, does not cause disease.**

Teacher Hints and Troubleshooting

1. The *E. coli* strain used for this lab must ferment lactose. These strains are usually called intestinal *E. coli.*

2. This experiment works best at 37˚C. At lower temperatures incubation times will be significantly longer.

3. *E. coli* culture needs to grow overnight prior to inoculating the student broth tubes. These cultures are good for only 1-2 days.

4. As gas is produced it should collect in the gas collecting tube. If the litmus milk inside the gas collecting tube is too solid to move, cracking of the curds is ample evidence of carbon dioxide production.

5. It is important that students understand that this a model system. We do not want students to think they can eat yogurt to provide lactase. In contrast, the commercial product, Lactaid, does provide the enzyme lactase.

6. Instructions for preparing yogurt are included at the end of this activity.

Tiny Bubbles Answer Key

1. State the problem you are investigating.

   What happens when E. coli *is grown in broth with and without lactose?*

2. Write a hypothesis using an if-then format.

   Students should develop a hypothesis or an answer to the problem. Many people use the "if...then..." form for writing a hypothesis. A good hypothesis will suggest how the experiment will be carried out and what to look for in the results. For the problem, one hypothesis could be that if the E. coli *bacteria are grown in a broth with lactose, they will produce carbon dioxide gas.*

3. What is the independent variable in this experiment? What is the dependent variable?

   Independent variable: Broth with lactose

   Dependent variable: Gas production

   These may vary depending on the student's hypothesis.

4. What conditions remain constant?

   In this experiment, everything is the same except one tube has lactose added to the broth. The tubes, the nutrient broth, and the procedure are held constant.

5. Describe the difference(s) observed between the two tubes. Sketch what you see.

   The nutrient broth tube should appear cloudy but will have no bubbles in the gas collecting tube.

   The lactose broth tube should appear cloudy and should show a definite accumulation of gas inside the gas collecting tube.

6. Write a short summary paragraph describing the difference in the two "test tube environments" and explain your results.

   The difference in the two "test tube environments" is the presence of lactose in one but not in the other. In the tube containing lactose, E. coli *ferments the sugar, resulting in the production of carbon dioxide gas.*

Tickled Pink Answer Key

Day One Observations				
	A. No bacteria	B. *Lactobacillus*	C. Mixed culture	D. *E. coli*
Gas present?	*no*	*no*	*no*	*no*
Color?	*purple*	*purple*	*purple*	*purple*
Texture?	*liquid*	*liquid*	*liquid*	*liquid*

Day Two Observations				
	A. No bacteria	B. *Lactobacillus*	C. Mixed culture	D. *E. coli*
Gas present?	*no*	*no*	*no*	*no*
Color?	*purple*	*pink*	*pink*	*purple*
Texture?	*liquid*	*solid*	*solid*	*liquid*

Day Three Observations				
	A. No bacteria	B. *Lactobacillus*	C. Mixed culture	D. *E. coli*
Gas present?	*no*	*no*	*no*	*yes*
Color?	*purple*	*pink*	*pink*	*pink*
Texture?	*liquid*	*solid*	*solid*	*liquid*

1. Did tube “A” (no bacteria) change from day two to day three? Explain.

   No, because this is the control tube with no microbial activity.

2. Litmus milk contains the indicator litmus that changes color to show the presence of an acid or a base. Pink indicates the presence of an acid and blue shows a base. On the pH scale, an acid has a pH value of less than 7 and a base has a pH value of over 7. Purple is neutral, indicating a pH of 7. On the table below, indicate the acid/base condition of each tube.

Test tube	Color	Explanation
A	*purple*	*control tube—no reaction, neutral*
B	*pink*	*acidic condition—lactic acid produced*
C	*pink*	*acidic condition—lactic acid produced*
D	*pink*	*acidic condition—lactic acid produced*

3. What can be determined about the pH from the color of the litmus milk in test tube “B” (*Lactobacillus*)?

   The microbes create an acidic environment.

4. How did the color of the litmus in test tube “C” (mixed culture) compare to the color in the other tubes? What does that indicate?

   This tube has a pink color. After the initial inoculation, Lactobacillus *broke down the lactose and produced an acidic environment. The* E. coli *did not change this.*

5. Summarize the data tables above comparing all tubes to each other. Be specific about results.

   Tube A is the control tube showing no color change or gas production over time in a sterile environment.

   Tube B shows that Lactobacillus *breaks down sugar and produces acid but no gas.*

   Tube C shows that in the mixed culture tube Lactobacillus *used the lactose; therefore* E. coli *did not produce gas.*

   Tube D shows E. coli *can break down sugar for food and in the process produces gas that was captured in the collecting tube.*

6. Does *Lactobacillus* produce gas when it ferments the lactose in milk?

   No

7. What happens when *E. coli* ferments lactose?

   One of the products of this reaction is a gas: specifically, carbon dioxide.

8. What will happen if *E. coli,* while living in the large intestine, finds lactose in the environment?

   The E. coli *will produce gas.*

9. Refer to tube "C" (mixed culture). If *Lactobacillus* are allowed to ferment milk **before** the milk is inoculated with *E. coli,* will *E. coli* be able to produce gas? Explain your answer.

   When the lactose is broken down by the Lactobacillus *first, there is no sugar left for the* E. coli *to ferment to produce gas.*

10. What part of the digestive process is not functioning properly in lactose intolerant individuals?

    The small intestine. This is the site of lactase production, the enzyme responsible for digesting lactose.

11. What substance in milk can be used by intestinal bacteria as a food source during fermentation?

    Lactose

12. *E. coli* and other bacteria live in the large intestine of **all** individuals. Why then do most individuals not suffer when they drink milk?

    Most individuals produce the enzymes needed to break down lactose in the small intestine. Therefore, by the time food reaches the large intestine (and the E. coli*) there is no sugar for the bacteria.*

13. What is the probable cause of the "rumbly tummy" that lactose intolerant individuals feel when they drink milk?

    The "rumbly" feeling is caused by the gas moving through the large intestine (colon).

14. Lactose intolerant individuals can often eat cultured milk products such as yogurt, buttermilk, or acidophilus milk with no complications. What did the bacteria do to the milk that now allows these individuals to eat the product without having symptoms?

    The bacteria have broken down the lactose in these products.

15. Design an experiment to test this question, "Is lactose broken down equally well by the bacteria found in different types of cultured milk products (i.e. yogurt, buttermilk, acidophilus milk)?" For this experiment, state the problem, form a hypothesis, list materials and protocols needed. If time permits, you may want to do this lab.

    Answers will vary.

Yogurt

The purpose of this lab is to make your own yogurt using milk and a small amount of starter (which is yogurt containing live microorganisms).

Yogurt is a fermented milk product. The combined action of two bacteria, *Lactobacillus bulgaricus* and *Lactococcus thermophilus* on the milk sugar (lactose) results in the production of acid (primarily lactic acid) which gives yogurt its sour taste. The acid acts on the milk protein (casein) causing changes in the structure of the protein (denaturation) which causes it to solidify (coagulate). This is similar to the heat denaturation (cooking) of egg white, which causes it to turn from a liquid to a solid. In summary, two bacteria ferment lactose to lactic acid in milk; the acid denatures the protein causing it to coagulate. These changes cause the milk to become semi-solid and give it the sour taste.

The bacteria that are used to make yogurt are heat-loving (thermophilic) organisms. They grow best at temperatures that kill most other microorganisms. In making yogurt it is important to kill undesirable bacteria and to provide growth conditions that are best for the desirable bacteria. Undesirable bacteria are killed by heating the milk to just above boiling (212°F, 100°C). All utensils should be very clean to prevent undesirable organisms from getting into the milk. Good growth conditions for the desirable bacteria include high temperature (130°F, 55°C) for growth and an environment that does not have a large amount of oxygen. To accomplish this, yogurt should be made in closed containers, kept as free from shaking as possible, and should be kept very warm. By sterilizing the milk and using clean utensils most undesirable organisms will be eliminated. By keeping the inoculated milk very warm even those few undesirable bacteria are prevented from growing (55°C is too hot for most of the undesirable bacteria). As the milk cools the yogurt bacteria will produce large amounts of lactic acid. The acid will prevent the growth of most bacteria (including the yogurt bacteria) so that by the time the milk cools to 37°C (99°F), which is a good temperature for many of the undesirable bacteria they will not be able to grow because of the acid. The acid and the lower temperature will also slow down and eventually stop the growth of the yogurt bacteria and no more acid will be made. It is best if the milk is kept hot enough to allow maximum growth of the yogurt bacteria but not enough to kill them. In order to keep the culture warm for as long as possible, the milk should be inoculated at about 55°C (130°F) and the culture should be insulated to retain heat. Once the yogurt is ready it must be kept refrigerated.

As long as the yogurt is always made from boiled milk and the cooking utensils are very clean (washed in very hot soapy water, rinsed in hot water, drained, and air dried; or washed in a dishwasher) the yogurt should contain virtually no undesirable bacteria. This yogurt can then be used as starter for the next batch of yogurt. Fresh yogurt must be made every 2-3 weeks, or the bacteria in yogurt will die and new yogurt must be bought to use as starter. If you make yogurt often and you are careful to keep it clean you should not ever have to buy yogurt again!

Making Yogurt in the Classroom

For steps #1-4, students will work in groups of four. For steps #5-8 students will work alone.

Materials (per group of 4)
720 mL of milk (made by mixing 180 g of powdered milk into 720 mL of skim, 2% or whole milk)
Yogurt starter (a container of commercial yogurt with active bacteria, such as Dannon)
Hot plate
Beaker in which to heat the milk
Spoons for stirring the milk
Thermometer
Styrofoam cup with lid (one per student)
Plastic bag (one per student)
Plastic wrap
Shredded newspaper or other insulating material (or an incubator)

1. Heat the milk in the beaker, stirring constantly (The yogurt will taste very bad if the milk is scorched). When the milk just begins to boil, turn off the heat. Cover with plastic wrap, allowing the plastic to touch the milk. This prevents a film from developing on the milk.

2. Allow the milk to cool to 57°C.

3. Remove the plastic wrap and stir milk. Pour milk into 4 Styrofoam cups (one for each student). Check temperature with thermometer.

4. When the milk in large cup cools to 55°C, add one teaspoon of the starter yogurt. Stir carefully, but thoroughly to distribute starter throughout the milk.

5. Cover the cup with plastic wrap and cover plastic wrap with plastic cup lid. Store the cup in a Ziploc bag surrounded with newspaper shreds to help retain heat.

   If an incubator is available, put yogurt cups into it at 37^{O}C.

6. Incubate 6 hours or longer (overnight is all right).

7. Remove cups. Check to see that milk is solid. Refrigerate.

8. If you want fruit and/or sugar in your yogurt, add it after the yogurt is cool.

   A fun treat is making yogurt smoothies. Add one styrofoam cup of yogurt, crushed ice, fruit and sugar (to taste) to a blender. Blend until well mixed.

Making Yogurt at Home

To make yogurt at home you need to modify the laboratory procedure. While you can make small quantities (such as 2 cups) it is better to make at least one quart and preferably 2 quarts. The larger volume will stay warm better.

Materials

Covered container of the size you want (1 or 2 quart Corning ware dishes are good; they retain heat well). It should be very clean.
Heavy-bottom pan (milk scorches easily in lightweight pans)
Wooden spoon
Candy thermometer
Small cup containing a teaspoon of yogurt starter (commercial yogurt such as Dannon, or you own previously made yogurt)
An old blanket folded to about 3 feet by 3 feet size, with several layers of newspaper

1. Heat milk (enough to fill container you want) to boiling, stirring frequently. You may use skim milk, whole milk, or 2% milk, but you should add one cup of powdered skim milk per 2 quarts of milk. If you want to use only powdered skim milk you should mix 1 part powder to 2 parts water (for example, 4 cups of water and 2 cups of powder).

2. Cool milk to 135°F and pour into container.

3. Mix a few tablespoons of 135°F milk into the starter in the small cup.

4. When milk is 130°F, pour starter-milk mixture into large container of milk. Stir thoroughly. Cover milk.

5. Place container in middle of newspaper-lined blanket. Fold newspaper around container so that it is well insulated. Wrap blanket over newspapers and put something on top to hold blanket closed.

6. Leave at least 6 hours or overnight if necessary. Check to see that milk is solid and refrigerate before eating.

7. As you remove yogurt from container smooth the surface so that “holes” don’t form. These “holes” will fill with whey if they are left.

   When yogurt is almost gone take out some for starter for next batch.

Dear Parents,

This week our science classes are discussing a rather common medical condition called lactose intolerance. Maybe you are familiar with this condition. Individuals who are lactose intolerant cannot drink milk or eat foods like cheese or ice cream without getting sick. The symptoms are various degrees of indigestion whenever milk-based foods are eaten. There are some commercial products around to reduce these symptoms. But what is really behind this condition? In our laboratory experiments this week your child will be trying to answer this and other related questions. Ask them what they are learning. Enjoy some quality time with your offspring over a big bowl of yogurt! If your family enjoys yogurt, why not try making your own? I have directions if you need them!

As always, you are welcome to come and visit to “get the scoop” on these science labs!