

Biotechnology Explorer™

**Microbes and Health Kit:
"What Causes Yogurtiness?"™**

Catalog #166-5030EDU

explorer.bio-rad.com

Store components of this kit at room temperature.

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BIO-RAD

Dear Educator,

The chemistry of the bacterial cell is brought into focus as students examine bacteria and their interaction with the environment. Enzyme catalyzed chemical reactions in bacteria provide energy for the bacteria as they change food into secreted waste products. In some cases, bacterial waste products can be the cause of disease symptoms and in other cases they may create foods and nutrients for people. Thus bacteria can sometimes be our friends and other times our foes. For a long time, biotechnology has utilized friendly bacteria in the production of foods such as cheese, sauerkraut, kimchi, coffee, sour cream, vinegar, sausage, and yogurt. Other bacteria cause cholera, typhus, leprosy, tuberculosis, and anthrax. In this lab students will examine both the risks and benefits of bacteria to better understand their role in disease and food production.

Discover the cause of disease. In the 18th century bacterial diseases were still a deadly mystery. Bacteria were sometimes found in diseased humans and animals — but did the bacteria cause the disease or did the bacteria merely follow a disease caused by another unknown agent? To know the cause is the first step toward cure or prevention. Join Robert Koch, Louis Pasteur, and the founders of modern microbiology in a thrilling search to find the bacterial culprit behind a new disease. The new disease examined in this lab is "yogurtiness" — an affliction of "healthy" milk that causes it to become acidic and thick. What is the cause of yogurtiness? Can you use Koch's postulates, the standard of proof in the identification of microbial disease agents, to identify the guilty microbe in this inquiry based activity?

Students will use microscopes, agar plates, and their powers of observation to identify the bacteria used to produce yogurt and to provide proof for their hypothesized identification. Use this kit to examine metabolism, cellular chemistry, and the role of bacteria in both disease and food microbiology.

This curriculum was developed in collaboration with Peggy Skinner of the Bush School in Seattle Washington. We would like to thank her for her invaluable guidance and contribution to this curriculum.

Ron Mardigian
Founder
Biotechnology Explorer Program

Create context. Reinforce learning. Stay current.

New scientific discoveries and technologies create more content for you to teach, but not more time. Biotechnology Explorer kits help you teach more effectively by integrating multiple core content subjects into a single lab. Connect concepts with techniques and put them into context with real-world scenarios.

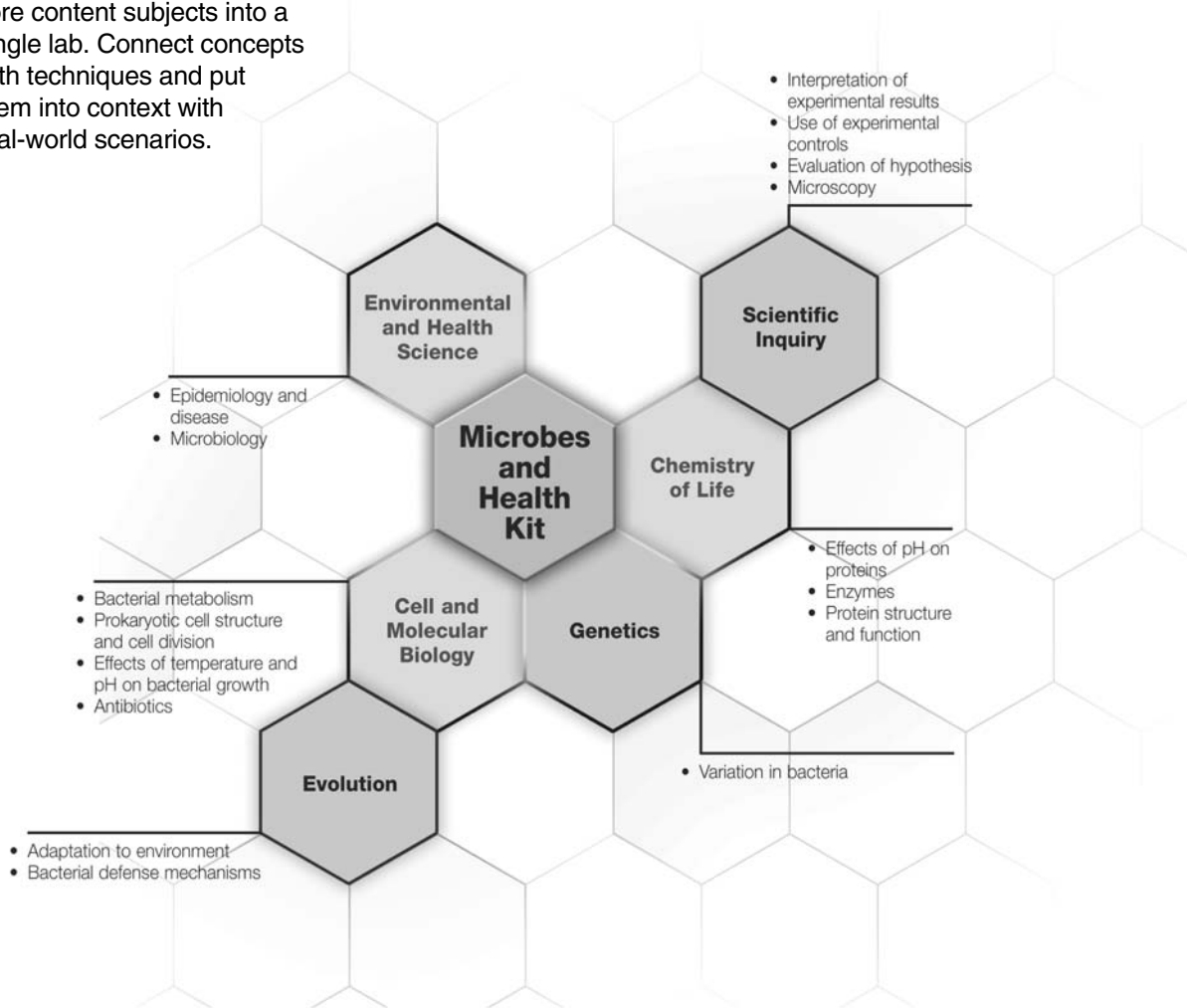


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Introduction

This lab activity uses simple techniques and common substances to demonstrate the discovery process of a disease-causing organism by following Koch's postulates. The lab also shows how bacterial cells take up food and use enzymatic reactions to gain energy by converting food into lactic acid.

Objectives

- To utilize Koch's postulates to find a causative agent for disease
- To practice microbial techniques
- To design a controlled experiment
- To reach a scientific conclusion from data and defend that conclusion

Robert Koch, a German physician who lived from 1843–1910, developed four basic principles to identify the causative agent for a particular disease. The postulates are:

1. The microorganism must be found in all organisms suffering from the disease, but not in healthy organisms.
2. The microorganism must be isolated from a diseased organism and grown in pure culture.
3. The cultured microorganism should cause disease when introduced into a healthy organism.
4. The microorganism must be again isolated from the inoculated, diseased experimental host and identified as identical to the original specific causative agent.

In this lab students will compare milk and yogurt physically and microscopically and identify bacteria in the yogurt as a possible cause of a condition called "yogurtiness". Yogurtiness is the condition of being like yogurt. They will then isolate and culture the bacteria from yogurt on agar plates and reintroduce the pure cultured bacteria into milk to see if they can induce yogurtiness. If the milk turns into yogurt they can then identify the bacteria and determine if it is the same bacteria as in their pure culture. Koch's postulates are used to conduct an experiment to determine the cause of yogurtiness. Milk will model the "healthy" individual and yogurt will model the "diseased" individual. Yogurtiness is caused by one or more strains of yogurt producing bacteria: *Streptococcus thermophilus* (*Streptococcus salivarius* subsp. *thermophilus*), *Lactobacillus bulgaricus* (*Lactobacillus delbrueckii* subsp. *bulgaricus*), *Lactobacillus acidophilus*, *Lactobacillus casei*, or *Bifidobacterium bifidum*. Of course, it is important to remember that yogurt producing bacteria do not cause any real disease and that yogurt itself is a very healthy and beneficial food.

Timeline

Pre-Lesson: Prepare agar plates 3–7 days ahead (30–60 minutes instructor preparation). Purchase milk and yogurt.

Lesson 1: Comparison of milk and yogurt. Inoculation of agar plates with yogurt followed by incubation at 37°C for 1–2 days (50 minute class, 30–60 minute instructor preparation).

Lesson 2: Identification of bacteria and inoculation of milk followed by incubation at 37°C for 1–2 days (50 minute class, 30–60 minute instructor preparation).

Lesson 3: Identification of bacteria from newly made yogurt (50 minute class, 15 minute instructor preparation).

Curriculum Fit

- Students develop abilities to conduct inquiry-based experiments
- Students formulate scientific hypothesis and conclusions using data, logic, and evidence
- Students develop an understanding of the role of microbes in disease and health
- Students learn microbiology skills commonly used in research
- Students gain knowledge of how cells break down food to form other products

Storage Instructions

All components of this kit may be stored at room temperature.

Safety Issues

The *Escherichia coli* bacteria HB101 K-12 strain contained in this kit is not a pathogenic organism like the *E. coli* strain O157:H7 that has sometimes been implicated in food poisoning. HB101 K-12 has been genetically crippled to prevent its growth unless grown on an enriched medium. However, handling of the *E. coli* K-12 strain requires the use of standard microbiological practices. These practices include, but are not limited to, the following: work surfaces are decontaminated once a day and after any spill of viable material; all contaminated liquid or solid wastes are decontaminated before disposal; all persons must wash their hands: (i) after they handle material containing bacteria, and (ii) before exiting the laboratory; all procedures are performed carefully to minimize the creation of aerosols; mechanical pipeting devices are used, mouth pipetting is prohibited; eating, drinking, smoking, and applying cosmetics are not permitted in the work area; wearing protective eyewear and gloves is strongly recommended. The *E. coli* bacteria are used in this kit only as a control and thus their use may be eliminated if there are concerns.

If an autoclave is not available, all solutions and components (loops and pipets) that have come in contact with bacteria can be placed in a fresh 10% bleach solution for at least 20 min for sterilization. A shallow pan of this solution should be placed at every lab station. No matter what you choose, all used loops and pipets should be collected for sterilization. Sterilize Petri dishes by covering the agar with 10% bleach solution. Let the plate stand for 1 hr or more and then pour excess plate liquid down the drain. Once sterilized, the agar plates can be double bagged and treated as normal trash. Safety glasses are recommended when using bleach solutions.

Ampicillin may cause allergic reactions or irritation to the eyes, respiratory system, and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. Ampicillin is a member of the penicillin family of antibiotics. Those with allergies to penicillin or any other member of the penicillin family of antibiotics should avoid contact with ampicillin.

Please refer to the Material Safety Data Sheets (MSDS) available from Bio-Rad by calling (800)-4BIORAD in the United States, or see www.bio-rad.com for further information on reagents in this kit. Please consult your local environmental health and safety regulations for proper disposal.

Do not eat the yogurt created in the laboratory. There are several websites in the additional information section with protocols for making yogurt if you would like to make yogurt for consumption.

Kit Inventory Checklist

This section lists the equipment and reagents necessary to conduct the microbes and health experiment in your classroom or teaching laboratory. We recommend student teams of 2–4 students per workstation. The kit contains reagents for 32 students working at 8 workstations made up of 4 students each.

Kit Components	Number/Kit	(✓)
Ampicillin	2 vials	<input type="checkbox"/>
LB nutrient agar powder (to make 500 ml)	1 pouch	<input type="checkbox"/>
Petri dishes, sterile, bags of 20	2 bags	<input type="checkbox"/>
Culture tubes, sterile, bags of 25	3 pks	<input type="checkbox"/>
Inoculation loops, sterile, 10 µl, packs of 10 loops	8 pks	<input type="checkbox"/>
<i>E. coli</i> HB101 K-12, lyophilized	1 vial	<input type="checkbox"/>
LB broth capsules, bags of 12 (to make 50 ml each)*	1 bag	<input type="checkbox"/>
Disposable plastic transfer pipets, packs of 10 pipets	1 pack	<input type="checkbox"/>

* LB broth capsules are included to extend the activity by using liquid cultures, if desired.

Required Accessories	Number/Kit	(✓)
Microwave or autoclave	1	<input type="checkbox"/>
Incubator at 37°C (Catalog #166-0501EDU)	1	<input type="checkbox"/>
500 ml graduated cylinder	1	<input type="checkbox"/>
1 L flask or bottle	1	<input type="checkbox"/>
Microscopes	1/workstation	<input type="checkbox"/>
Microscope slides and cover slips	40 slides/80 cover slips	<input type="checkbox"/>
pH paper (pH range 4–7 or wider)	48 pieces	<input type="checkbox"/>
Permanent marker pens	8	<input type="checkbox"/>
Table sugar (sucrose)	10 grams	<input type="checkbox"/>
Toothpicks or micropipet tips	box	<input type="checkbox"/>
Distilled water	1 L	<input type="checkbox"/>
Milk	400 ml	<input type="checkbox"/>
Plain cow's milk yogurt (2–4 brands, must be labeled as containing live and active cultures – the latest available expiration date is preferred)	100 ml	<input type="checkbox"/>

Optional Accessories

Magnifying glasses (to view bacterial colony morphology)

Refills Available Separately

Microbes and Health kit refill package, (Catalog #166-5021EDU) includes Ampicillin, 12 LB broth capsules, LB nutrient agar powder, and HB101 K-12 *E.coli*

LB nutrient agar powder (Catalog #166-0600EDU) 1 package

Ampicillin (Catalog #166-0407EDU) 1 vial

E. coli strain HB101 K-12 (Catalog #166-0408EDU) 1 vial

Background for Instructors

Bacteria Are Everywhere

Bacteria are the single most successful form of life on the Earth. There are probably more bacteria, more species, and more total biomass of bacteria than any other lifeforms. Bacteria are found in soil, water, in and on animals, in and on plants, in and on humans, and even miles below the ground. There is speculation that bacteria or similar forms of life may exist on Mars or other planets.

Characteristics of Bacteria

Bacteria are one of the three great domains of life along with Eukarya (Animals, Plants, and Fungi) and Archaea (ancient bacteria-like organisms classified as a separate domain of life by Carl Woese in 1977). Bacteria and Archaea are classified as prokaryotes, single-celled creatures usually too small to be seen by the unaided eye. Bacteria are so small that it would take 5,000 to 50,000 in a row to stretch for an inch. Bacteria have no separate compartment (nucleus) to hold their DNA as eukaryotes do. Bacteria can sometimes, but not always, move by tiny tails called flagella. Bacteria sometimes grow connected to other bacteria forming chains. Some types of bacteria that may be seen in this lab grow connected in chains.

Bacteria as Pathogens

When we think of bacteria we usually think of disease. In fact, only a tiny minority of bacteria are capable of causing disease. Bacteria that do cause disease have played an enormous role in the history of humanity—cholera, typhus, the bubonic plague, tuberculosis, and other bacteria have sickened and killed millions. The development of antibiotics has greatly reduced the dangers of bacterial diseases. However, due to the overuse of antibiotics some bacterial strains (such as methicillin-resistant *Staphylococcus aureus* or MRSA) have developed antibiotic resistance leaving humanity exposed to the reemergence of old bacterial threats.

Bacteria can also spoil food such as milk. Milk is an ideal growth medium for bacteria and may contain both spoilage bacteria capable of souring milk, and pathogenic bacteria which might cause disease in humans, such as brucellosis, bovine tuberculosis, and scarlet fever. Milk is pasteurized by heating to 62.9°C for 30 min, or 71.6°C for 15 sec, and then cooling rapidly. Pasteurization destroys all pathogenic bacteria, and most but not all, spoilage bacteria. Thus milk still needs to be kept cool when stored. Grade A milk should contain less than 30,000 bacteria per milliliter.

History of Bacteriology

Anton van Leeuwenhoek of the Netherlands first saw bacteria through a microscope in 1676 and called them animalcules (tiny animals). Later Christian Gottfried Ehrenberg coined the term “bacterium” (meaning “small staff” in Greek) in 1828. In 1835 Agostino Bassi proposed the “germ theory of disease” which connected the spread of disease to unseen microorganisms, as previously bacteria were thought to arise spontaneously in suitable environments. Louis Pasteur and John Tyndall showed that boiled broth grew bacteria only when exposed to the air thus disproving the theory of spontaneous generation. In 1875 Robert Koch was able to offer convincing proof of the germ theory by proving that anthrax was caused by bacteria. Koch’s set of rules (Koch’s postulates) for proving the cause of anthrax are the basis for assigning the cause of disease to a particular microbe. The postulates are also the basis for the experiments in this lab.

Types of Bacteria and Bacterial Colonies

There are several distinct morphologies or shapes of bacteria. The three major shapes are coccus (spherical), bacillus (rod-shaped), and spirillum (spiral). Cocci and bacilli can exist singly, in pairs (diplococci or diplobacilli), attached in long strings (streptococci or streptobacilli), or connected in other arrangements (staphylococci or staphylobacilli). There are various forms of spiral bacteria too, such as comma-shaped (*Bdellovibrio*), helical (*Helicobacter pylori*), or long twisted spirochete forms. It is best to examine fresh cultures as older bacteria are occasionally oddly shaped and may have lost motility.

Bacteria increase in number by binary fission (splitting in half). Some bacteria can divide every 15–20 minutes! A single bacterium on a solid medium, such as an agar plate, increases logarithmically so that overnight a single bacterium becomes millions or billions. These millions or billions of bacteria form a visible "colony" on an agar plate. A colony of bacteria can itself have a distinct form and be large or small. Some bacterial colonies are so small that they cannot be seen with the unaided eye. Colonies may be circular, irregular, or branching. The edge of the colony may be smooth, wavy or serrated. The colony may be flat, raised or raised only in the center.

Bacteria are also differentiated by their cell walls. Some have thick cell walls made of peptidoglycan molecules. The cell walls of these bacteria take up a dye called Gram stain and thus are called Gram-positive bacteria. Other bacteria have thinner cell walls that do not absorb Gram stain and thus are called Gram-negative bacteria. The lactic acid bacteria found in yogurt are Gram-positive bacteria. The HB101 K-12 *E. coli* bacteria provided in this kit are Gram-negative bacteria.

Bacterial Metabolism

Like all living things bacteria require food, often in the form of sugars, to gain energy. Bacteria break down sugars chemically into other molecules using enzymes. Enzymes are large proteins that speed up chemical reactions. This process of bacterial metabolism is often called fermentation.

Some bacteria require oxygen from the air to grow and are called aerobes. Other bacteria grow only in the absence of oxygen and are called anaerobes. Some bacteria can grow either with or without oxygen and are referred to as facultative anaerobes. Aerobic bacteria use oxygen to break sugar into intermediate products and then finally into carbon dioxide and water. Lacking oxygen, anaerobic or facultative anaerobic bacteria usually do not reduce sugars completely to carbon dioxide and water. Often these bacteria convert sugar into pyruvic acid and then convert the pyruvic acid into other by-products.

Yogurt forming bacteria are anaerobes and break down milk sugar (lactose) into pyruvic acid and then into lactic acid using enzymes. Lactic acid is the by-product or waste product made by lactic acid bacteria. Lactic acid also lowers the pH of milk making it acidic. The acidic conditions cause casein (a common protein in milk) to denature (or curdle) and become more solid. In addition the acidic conditions inhibit the growth of other microorganisms that might spoil the yogurt. Thus lactic acid causes the yogurt to stay fresh, while at the same time remaining digestible by people who can break lactic acid down for additional energy. Other bacteria can break down sugars and pyruvic acid and make other by-products. The *E. coli* bacteria break sugar down into succinic acid, ethanol, acetic acid, formic acid, and lactic acid.

Koch's Postulates

By the mid-19th century, the famous French scientist Louis Pasteur had conducted extensive studies on the role of bacteria in fermentation, and had shown conclusively that germs did not spontaneously appear in susceptible hosts (spontaneous generation), but rather needed to come in contact with the host first. There was already a prevailing assumption at the time that microbes were in some way connected with disease, but whether their presence was the cause or just a result of disease was unclear. Furthermore, many infected tissues contained more than one type of microorganism. This made it difficult to define with certainty the role any particular type of bacterium played in disease. The work of Pasteur and others, along with improved techniques in microscopy and the discovery of semi-solid culture media, all paved the way for the work of Robert Koch.

Koch had been studying anthrax, a deadly disease that infects both humans and animals, and he noticed that certain rod-shaped bacteria and their spores were characteristically found in the tissues of sick sheep. He meticulously isolated these bacteria, which he named *Bacillus anthracis*, and grew pure cultures in a medium consisting of the aqueous humor of cattle or rabbit eyeballs. Next, he introduced the bacteria from the cultures into healthy rabbits. When the rabbits subsequently developed symptoms of anthrax, Koch again isolated the bacteria from the rabbit tissue and observed them under the microscope to confirm that they were indeed the same ones he had seen in his original culture.

The steps he used are now known as "Koch's postulates." Meeting the criteria laid down by Koch is referred to as "satisfying Koch's postulates" and is considered the standard evidence required to show that a microorganism causes a particular disease.

To demonstrate Koch's postulates, students must do the following:

- Describe and record the symptoms shown
- Isolate and identify the suspected pathogen from the infected material and establish a pure culture
- Use the pure culture to infect new material. Describe and record the symptoms shown by the material. Check that these are the same as their original observations
- Again isolate and identify the organism

Beneficial Bacteria and Yogurt

Despite our longstanding association of bacteria with disease, most bacteria are essentially harmless. In fact, many bacteria are beneficial. Bacteria break down waste organic material. *Rhizobium* bacteria take nitrogen from the air and convert it into a usable form (fixation). Intestinal bacteria break down indigestible material and synthesize nutrients. Some types of bacteria are necessary for the manufacture of certain food products, such as cheese, kimchi, sour cream, pickles, and yogurt.

Yogurt is made by adding specific strains of bacteria into milk, which is then fermented under controlled temperatures and environmental conditions. The bacteria ingest natural milk sugars and release lactic acid as a waste product thus making the milk acidic. The increased acidity causes casein (the most common milk protein) to tangle into a solid mass (called curd) in a process called denaturation. The increased acidity (the usual pH of yogurt is 4–5) also inhibits the growth of other dangerous bacteria. To be classified and sold as yogurt in the United States it is required that yogurt must contain the bacteria strains *Streptococcus thermophilus* (*Streptococcus salivarius* subsp. *thermophilus*) and *Lactobacillus bulgaricus* (*Lactobacillus delbruecki* subsp. *bulgaricus*). Often these two are cocultured with other lactic acid bacteria for taste or health effects including *Lactobacillus acidophilus*, *Lactobacillus casei*, or *Bifidobacterium*

bifidum. In most countries, a product may be called yogurt only if live bacteria are present in the final product. A small amount of live yogurt can be used to inoculate a new batch of yogurt, as the bacteria reproduce and multiply during fermentation. Pasteurized products, which have no living bacteria, are called fermented milk. In the United States yogurt must contain at least a billion viable bacteria per gram at the time of manufacture and at least a million viable bacteria per gram at the expiration date.

Yogurt has nutritional benefits beyond those of milk—people who are lactose intolerant often enjoy yogurt without ill effects, apparently because live yogurt cultures contain enzymes which help break down lactose inside the intestine. Yogurt also has medical uses, in particular for a variety of gastrointestinal conditions, such as preventing antibiotic-associated diarrhea.

In this lab students will isolate the bacterial strains found in a yogurt sample on agar in a petri dish, then use those same strains to inoculate fresh milk to find out if they can reproduce the same yogurt. Students should be able to conclude that the acidic, solidified nature of yogurt is caused by bacteria acting upon milk.

Antibiotics

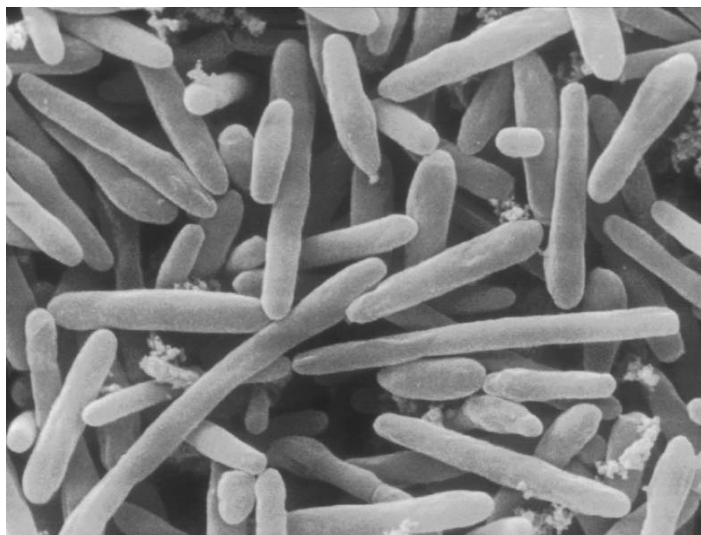
Early attempts to treat bacterial infections sometimes employed substances, such as arsenic or strychnine, that were nearly as toxic to humans as to bacteria. In 1928 Alexander Fleming discovered penicillin, a compound produced by mold, that inhibited the growth of bacteria without serious harmful effects upon humans. Many different types of antibacterial antibiotics have been discovered since that time. These antibiotics have vastly reduced the incidence of bacterial disease. Modern society has almost forgotten how great the dangers of bacterial disease once were. Careless misuse of antibiotics now threatens a return of potent bacterial diseases. Massive amounts of antibiotics are used in animal feed inadvertently selecting for the growth of bacteria resistant to many classes of antibiotics. People often needlessly take antibiotics for viral infections – again selecting for the growth of antibiotic resistant bacteria. In addition patients often discontinue use of antibiotics as soon as they feel better leaving the most resistant bacteria in place to start a new round of infection.

Antibacterial antibiotics are either bactericidal (kill bacteria) or bacteriostatic (prevent bacteria from dividing). There are many different modes of action for antibiotics. For instance, some inhibit the function of important enzymes present only in bacteria and not in mammals. Others destroy components of bacterial cell walls that are not used in mammalian cells.

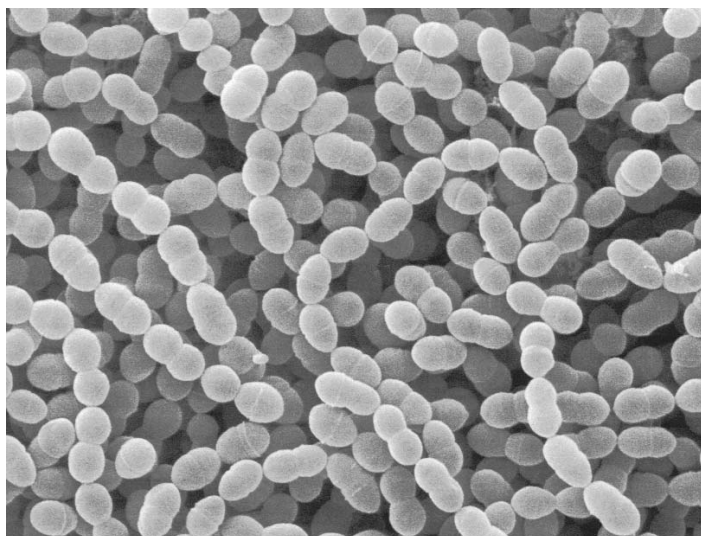
The antibiotic ampicillin is included in this kit both as an additional control and as a tool to allow further experimentation. Ampicillin is a beta-lactam antibiotic similar to penicillin and amoxicillin. It inhibits Gram-positive bacteria and some Gram-negative bacteria, such as *E. coli*, and it acts by preventing the synthesis of bacterial cell walls eventually leading to the death of the bacteria. Ampicillin is widely used in molecular biology as a selective agent since the gene for resistance to ampicillin (encoding for the beta-lactamase enzyme) can be inserted into bacteria on plasmids that may also carry genes of interest to scientists. Those bacteria that survive on ampicillin containing media will also have the gene of interest.

Sterile Technique

When culturing bacteria it is important to avoid contamination. Contaminating bacteria and molds are found everywhere, including on hands and lab benches, so it is important to avoid these surfaces. The round circle at the end of inoculating loops and the surfaces of agar plates should not be touched or placed onto potential contaminating surfaces. Wipe down lab benches with 70% alcohol or a 10% bleach solution wearing appropriate safety equipment.



Lactobacillus bulgaricus
(rod-shaped)



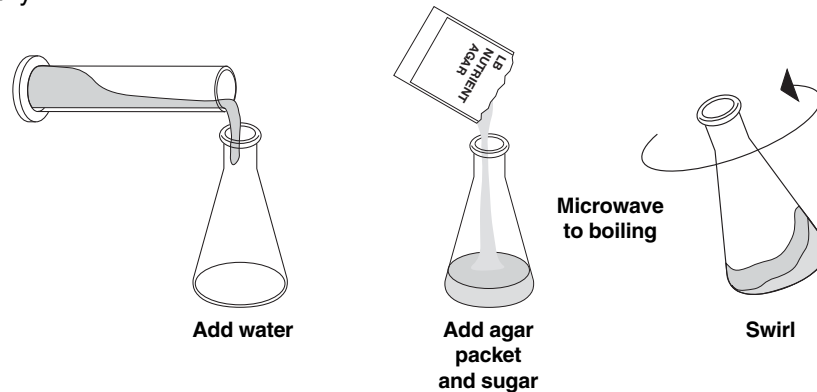
Streptococcus thermophilus
(spherical-shaped)

Instructor's Advance Preparation

These instructions are designed for 8 workstations with up to 4 students per workstation.

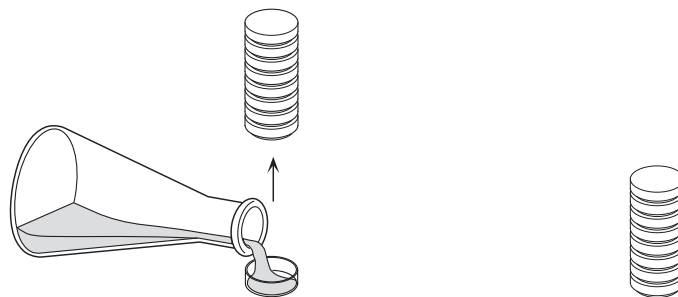
Lesson 1 Advanced Preparation Step 1: 3–7 days prior to laboratory

1. Prepare LB sugar agar plates as below.
2. To prepare one package of LB nutrient agar, add 500 ml of distilled water to a 1 L or larger Erlenmeyer flask. This should make enough agar for 30–40 plates.
3. Add the entire contents of one LB nutrient agar packet. Add 5 g of table sugar (sucrose). Swirl the flask to dissolve the agar and sugar. Heat the agar to boiling in a microwave.
4. Repeat the heating and swirling cycle about three times until all the agar is dissolved. There should be no more clear specks swirling around. Be careful to allow the flask to cool a little before swirling so that the hot medium does not boil over onto your hand.
5. Once all the agar has been dissolved, allow the agar to cool so that the outside of the flask is just comfortable to hold (50°C). Be careful not to let the agar cool so much that it begins to solidify.



Pouring Plates

1. Label the outside of the bottom plate with date and "LBS".
2. Stack the empty plates 4-8 high. Starting with the bottom plate lift the lid and the plates above straight up and to the side with one hand and pour the agar with the other.
3. Fill the plate about one-third to one-half (~10 ml) with agar, replace the lid and continue up the stack. Once the agar cools label the bottom plate "LBS" and record the date.
4. After the plates have dried for two days at room temperature they can be used or stacked and stored in the original plastic sleeve to prevent dehydration. The stack is inverted, the sleeve taped shut, and the plates stored upside down in the refrigerator until used.



Lesson 1 Advanced Preparation Step 2: 24 hr or less prior to lesson 1 laboratory

1. Prepare sterile water

Add approximately 100 ml of distilled water to a loosely capped bottle. Heat until boiling using a microwave or hot plate. Simmer for 10 minutes to sterilize using a low power setting on the microwave or hot plate. Alternatively use an autoclave. Seal container and allow to cool.

2. Rehydrate *E. coli* HB101 K-12 bacteria

Using a sterile pipet, rehydrate the lyophilized *E. coli* HB101 K-12 by adding 250 µl of sterile water directly to the vial. Recap the vial and shake to mix. Store the rehydrated bacteria in the refrigerator until used (preferably within 24 hours).

3. Obtain samples of milk and yogurt

- Purchase milk and 2–4 brands of fresh plain cow's milk yogurt labeled as containing live and active cultures and having the freshest best before date. Store in refrigerator.
- Label 8 culture tubes "milk" and aliquot approximately 5 ml of milk into each milk tube. Store in refrigerator until use.
- Optional: Aliquot the yogurt into culture tubes or small labeled cups or beakers to distribute between groups. The exact volume is not important. Alternatively have students use yogurt directly from the yogurt container. Store in refrigerator until use.

Student Workstations

Materials Needed for Each Workstation	Provided by	Quantity/workstaion
Microscope	Instructor	1
Microscope slides	Instructor	2
Cover slips	Instructor	4
pH paper (pH range 4-7 or wider)	Instructor	3 pieces
Cup with 5 ml yogurt*	Prepared by instructor	1
Culture tube with 5 ml milk	Prepared by instructor	1
LB sugar agar plates	Prepared by instructor	3
Sterile inoculation loops	Kit	3
Toothpicks or micropipet tips	Instructor	3
Marker pen	Instructor	1
Sterile water	Instructor	1 ml

Common Workstation	Quantity
Vial with <i>E. coli</i> rehydrated on ice	1

* Distribute the different brands of yogurt between the groups and have groups examine the properties of different yogurt brands. Each group should label the tube with initials so it can be saved for subsequent lessons.

Lesson 2 Advanced Preparation: 24 hr or less prior to lesson 2 laboratory

1. Prepare scalded milk
 - a. Add approximately 300 ml of milk to a beaker or pan and heat to just boiling/bubbling (86–93°C) and then turn off heat and allow to cool—store in the refrigerator for periods over two hours. This can be done with care in a microwave using a low power setting. Take care that the milk does not boil over onto your hand.
 - b. Aliquot approximately 5 ml into 48 culture tubes (6 per workstation). Store in a refrigerator.
2. Rehydrate Ampicillin

Ampicillin is shipped freeze-dried in a small vial. Add 1 ml of sterile water directly to one vial to make a 30 mg/ml ampicillin solution. Recap the vial and shake to mix. Label the vial with the concentration of ampicillin. Rehydrated ampicillin can be stored in the refrigerator for up to 1 month and in the freezer for up to 1 year.

Student Workstations

Materials Needed for Each Workstation	Provided by	Quantity/workstation
Microscope	Instructor	1
Microscope slides	Instructor	3–6
Cover slips	Instructor	3–6
pH paper (pH range 4-7 or wider)	Instructor	2 pieces
Cup with 5 ml yogurt (from lesson 1)	Instructor	1
Culture tube with 5 ml scalded milk	Prepared by instructor	6
Inoculated LB sugar agar plates	From lesson 1	3
Sterile inoculation loops	Kit	3
Magnifying glass (if available)	Instructor	1
Sterile loops	Instructor	4
Sterile water	Instructor	1 ml
Marker pen	Instructor	1
Toothpicks or micropipet tips	Instructor	6

Common Workstation	Provided by	Quantity
Vial with rehydrated ampicillin (on ice)	Instructor	1

Lesson 3 Advanced Preparation

Student Workstations

<u>Materials Needed for Each Workstation</u>	<u>Provided by</u>	<u>Quantity/workstation</u>
Cultured milk tubes	From lesson 2	6 tubes
Microscope	Instructor	1
Microscope slides	Instructor	4
Cover slips	Instructor	8
Yogurt plates	From lesson 2	1
pH paper (pH range 4-7 or wider)	Instructor	6 pieces
Toothpicks or micropipet tips	Instructor	box

Quick Guide

Lesson 1

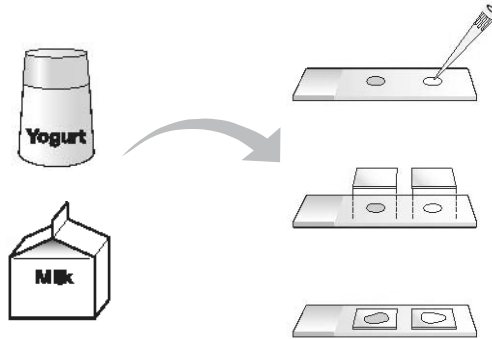
Postulate 1: Identify possible pathogens

1. Compare yogurt and milk with respect to appearance, smell, and pH. Record observations.



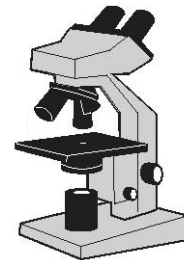
2. Label left hand edge of slide "yogurt" and right hand edge "milk".

3. Dip toothpick in yogurt, mix with a drop of water on left hand side of slide, and cover with cover slip.



4. Add drop of milk to right hand side of slide and cover with cover slip.

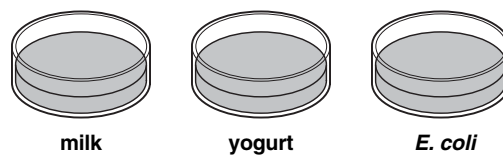
5. Observe yogurt and milk under the microscope. Describe and draw what you see.



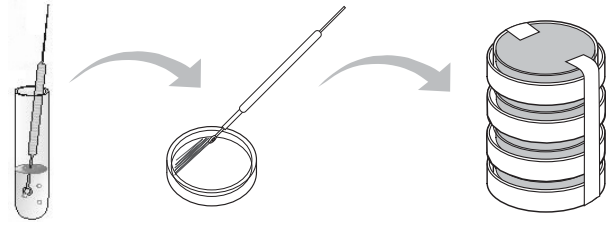
6. Repeat steps 1–5 with a different brand of yogurt.

Postulate 2: Isolate and culture suspected pathogens

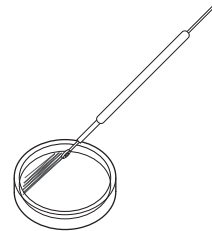
7. Label 3 LB sugar agar plates on the bottom (not the lid) with your initials and one as "milk", one as "yogurt", and the third as "*E. coli*".



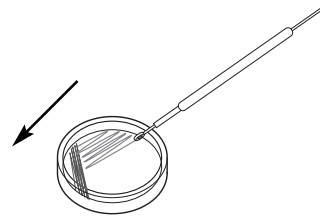
8. Streak milk onto milk plate for single colonies. Streak yogurt onto yogurt plate for single colonies as above. Streak *E. coli* onto *E. coli* plate for single colonies as above.



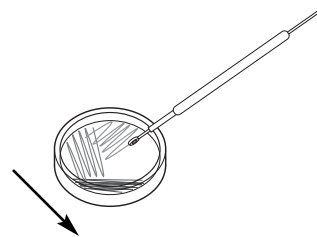
- A) Streak for single colonies by gently rubbing the loop back and forth in the top left corner of the plate about 10 times. Stay in the top left quadrant of the plate and do not break the surface of the agar.



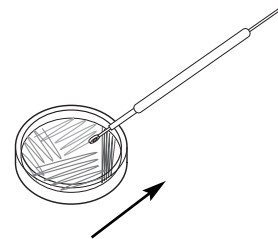
- B) Rotate the plate 45° and using the same loop draw the loop through one end of the first streak. **Do not dip the loop back into the starting material.** Then rub the loop back and forth in the second quadrant about 10 times. Avoid passing the loop into the first streak.



- C) Rotate the plate 45° and using the same loop draw the loop through one end of the second streak and rub the loop back and forth in the third quadrant about 10 times. Avoid passing the loop into the first and second streaks.



- D) Rotate the plate 45° and using the same loop draw the loop through one end of the third streak and rub the loop back and forth in the fourth quadrant about 10 times avoiding all previous streaks.



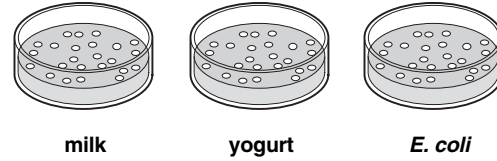
9. Invert the plates and place in incubator at 37°C for 24–48 h.



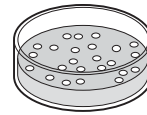
Lesson 2

Postulate 2 continued: Isolate and culture suspected pathogens

1. Obtain plates from previous lesson. Count the individual colonies on each plate. Record results.

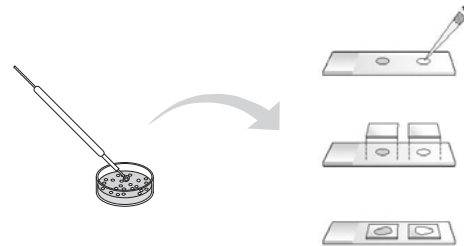


2. Observe colonies. Use a magnifying glass if available. Record how many different types of colonies you have on each plate. Use a marker to circle one of each type of colony and label with a number on the bottom of the plate.



3. Describe the appearance of each numbered colony.

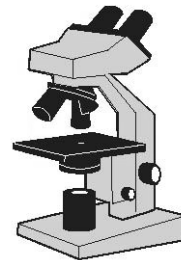
4. Label some slides according to your colony numbers. Use one slide for two samples as in the first lesson.



5. Pick a numbered colony from the yogurt plate, mix with a drop of water on right hand side of the appropriately numbered slide, and cover with a cover slip.

6. Repeat with the other numbered colonies from the yogurt, milk, and *E. coli* plates.

7. Observe colonies under the microscope. Describe and draw what you see.

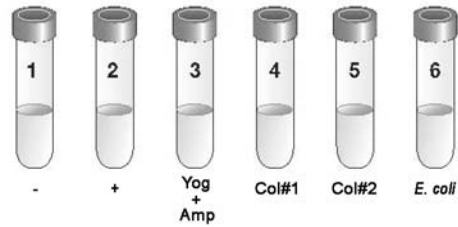


8. Compare the bacteria with your descriptions of those observed in the yogurt in the first lesson.

Postulate 3: Inoculate healthy individual with pure culture of suspected pathogen

9. Label 6 tubes of milk as follows:

- Tube 1 Negative control
- Tube 2 Yogurt (positive control)
- Tube 3 Yogurt + amp
- Tube 4 Yogurt Colony #1
- Tube 5 Yogurt Colony #2
- Tube 6 *E. coli*



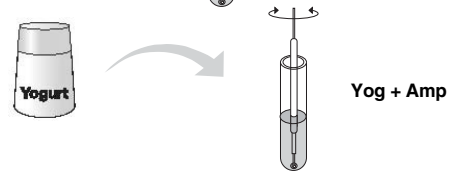
10. Add 10 µl or 1 drop of ampicillin to tube "Yogurt + amp".



11. Dip a fresh inoculation loop into the yogurt and swirl the loop into tube "positive control".

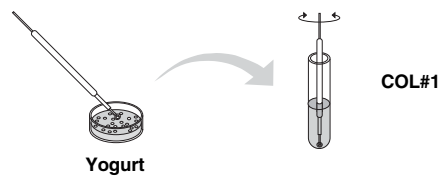


12. Use the same loop to dip into the yogurt again and swirl into the "Yogurt + amp" tube.



13. Identify two colonies on the yogurt agar plate that you investigated in the previous lesson of different types, if possible. Number the colonies 1 and 2 on the bottom of the plate and record which is which. If there is only one type of colony on your yogurt plate then number two similar colonies.

14. Using a fresh inoculation loop, pick colony #1 and transfer it to the tube "yogurt colony #1".



15. Using a fresh inoculation loop, pick colony #2 and transfer it to the tube "yogurt colony #2".



16. Using a fresh inoculation loop, pick an *E. coli* colony and transfer it to the tube "*E. coli*".



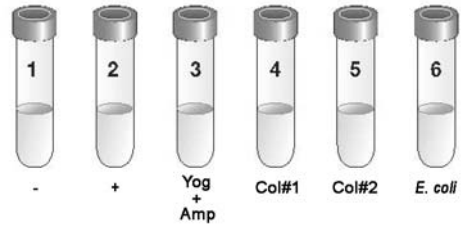
17. Place the tubes in an incubator or water bath at 37°C for 24–48 h.

QUICK GUIDE

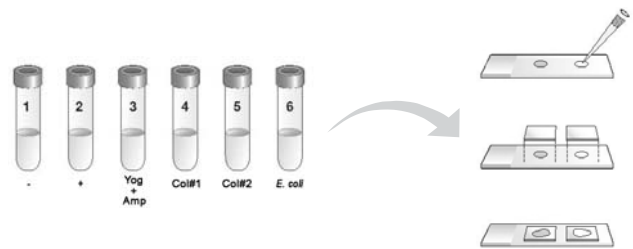
Lesson 3

Postulate 4: Isolate and identify suspected pathogen from newly diseased individual

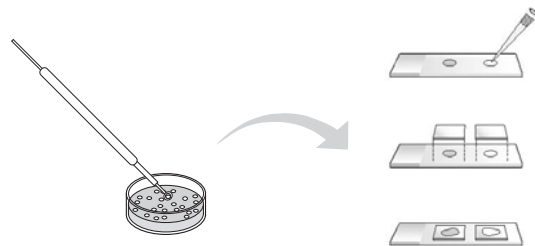
1. Obtain milk tubes and yogurt agar plate from previous lesson. Describe each milk culture with respect to appearance, smell, and pH.
2. Label 3 slides according your milk tube labels. Use one slide for two samples on the right and the left as in the first lesson.
3. Label a fourth slide yogurt colony #1 on the right and yogurt colony #2 on the left.



4. Prepare slide samples of each milk culture for viewing under microscope as in previous lessons. For solid cultures, dip toothpick in culture and mix with a drop of water. For liquid cultures, add a drop to the slide. Cover with cover slip.

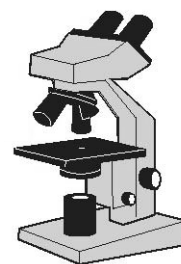


5. Pick a colony from the yogurt plate similar to that used to start the yogurt cultures in tube 4 (i.e. the same colony type as yogurt colony #1). Mix colony with a drop of water on right hand side of the appropriately numbered slide and cover with cover slip. Repeat with yogurt colony #2 on the left of the slide.



6. Observe slides under the microscope. Describe and draw what you see.

7. Using the microscope compare any bacteria in the newly infected cultures in milk tubes 4 and 5 with the pure bacteria used to inoculate these cultures. Are they the same?



Student Manual

Background

In the 1800's microbial diseases were a terrifying mystery. People sickened and died without apparent cause. It had long been suspected that contact with an infected individual was necessary for the transmission of disease, but this was not true for all diseases. Early microbiologists acted as detectives on the trail of a multitude of microbial killers. They were able to view bacteria from diseased individuals with microscopes, but how could they prove that the bacteria actually caused the disease? We will use Koch's postulates, a series of tests devised by Robert Koch, a German physician of the 1800s. Koch's postulates are widely used to prove that a particular microbe causes a particular disease.

Koch's postulates:

1. The microorganism must be found in all organisms suffering from the disease, but not in healthy organisms.
2. The microorganism must be isolated from a diseased organism and grown in pure culture.
3. The cultured microorganism should cause disease when introduced into a healthy organism.
4. The microorganism must be again isolated from the inoculated, diseased experimental host and identified as identical to the original specific causative agent.

Since it is dangerous and often unethical to experiment on humans, scientists often use model systems to simulate diseases in humans. Frequently, medical researchers will examine diseases in animals so that they can learn more about similar diseases in humans. You will use a model to test Koch's postulates. In this model, milk will represent a healthy individual. At times milk will develop a condition that causes it to thicken and turn into yogurt. This is the "yogurtness disease." You will play the role of a medical investigator from a time over a hundred years ago. You suspect that the yogurtness disease may be caused by something that is found in yogurt. You will use Koch's postulates to prove or disprove the hypothesis that microbes found in yogurt are the cause of yogurtness disease. Of course it is important to remember that real yogurt is a very healthy food and that any microbes found in yogurt are harmless and do not cause disease in healthy humans. Only a small minority of any bacteria cause disease in humans. In fact the "probiotic" (beneficial) bacteria found in yogurt may be helpful for digestion and may promote good health.

Ampicillin may cause allergic reactions or irritation to the eyes, respiratory system, and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. Ampicillin is a member of the penicillin family of antibiotics. Those with allergies to penicillin or any other member of the penicillin family of antibiotics should avoid contact with ampicillin.

Pre-lab Focus Questions

1. What diseases do you know of that are caused by bacteria?

2. What diseases do you know of that are not caused by bacteria?

3. What characteristics allow bacteria to cause diseases?

4. How are bacterial diseases treated?

5. How can the spread of bacterial diseases be prevented?

6. Are all bacteria harmful? If not, describe the benefits of some bacteria.

Lesson 1: Postulate 1

Identify Possible Pathogen

Compare the healthy individual (milk) and the diseased individual (yogurt) for different properties.

1. Describe the differences between the milk and yogurt in texture, smell, color, and any other observable characteristics. Also test the pH of the milk and each type of yogurt. Your instructor will have directions for use of the pH indicator.



Record the differences between yogurt and milk in Table 1 below. Different groups will have different types of yogurt – share your samples if possible. Examine more than one type of yogurt. Are there any attributes that are common to both types of yogurt but not milk?

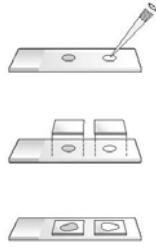
Table 1. Milk and yogurt characteristics.

Characteristics	Milk	Yogurt 1	Yogurt 2
Texture			
Color			
Smell			
pH			
Other Observations			

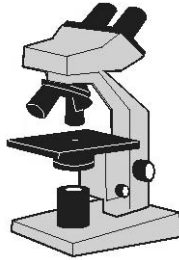
Examine the milk and yogurt under the microscope.

2. Label the left side of a microscope slide "yogurt" and the right side "milk."
3. Use a toothpick or micropipette tip to place a small amount of yogurt on the left side of the slide. The yogurt should come from below the surface if possible. Add one drop of sterile water and mix with a toothpick or micropipette tip. Place a cover slip over the top of the mixture.

4. On the right side add a drop of milk and place a cover slip over it.



5. Examine each sample under the microscope at 400x magnification. It may be necessary to adjust the condenser lens on the microscope to get the best image of the bacteria. Describe your observations for both the milk and yogurt in Table 2. Describe what you see at different magnifications on the microscope – recording the magnification and what you observe.



At a magnification of 400x bacteria should be visible and may be observed to have different shapes. Some may be spheres and others may be rod-shaped. Some may be linked together in chains. In Table 3 draw any microbes you see.

6. Examine a different brand of yogurt in the same manner. Describe and draw what you observe in Tables 2 and 3.

Table 2: Descriptions of milk and yogurt under the microscope.

Milk	Yogurt 1	Yogurt 2

Table 3: Drawings of microbes seen under the microscope.

Milk	Yogurt 1	Yogurt 2

Lesson 1 continued: Postulate 2

Isolate and culture the suspected pathogen

- Label 3 LB sugar agar plates on the bottom (not the lid) with team names or initials and label one plate "milk", one plate "yogurt" (and the brand of yogurt), and one plate "*E. coli*".



- Dip a sterile inoculation loop into the milk, check that you have a film of liquid over the loop, and streak the milk over the "milk" plate using the method on the next page.

Use a fresh loop to obtain a dab of yogurt from beneath the surface and streak it on the "yogurt" plate. Use as little yogurt as possible and follow the method shown on the next page.

With another fresh sterile loop, streak a loop of *E. coli* on the "*E. coli*" plate.

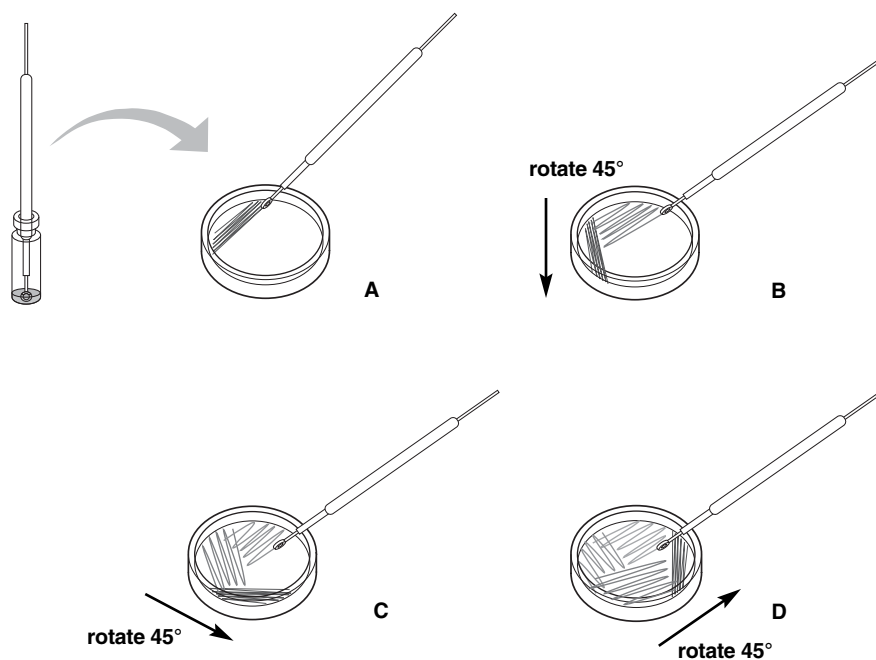
Streaking Plates

Streaking is done to make single colonies from concentrated bacteria. Each colony grows from one bacterium and thus a colony is a "clone" or group of genetically identical individuals. A tiny drop of the original bacterial suspension contains millions or billions of individual bacteria and must be diluted multiple times to isolate a single bacteria. Under favorable conditions *E. coli* can double every 20 minutes and thus a single bacterium will multiply to become billions of genetically identical cells in less than 24 hours.

- Insert a sterile inoculation loop into a bacterial colony, yogurt, or milk. Insert the loop straight into the container without tilting. Remove the loop and gently rub it back and forth over the agar in the top left-hand corner as shown on the next page. The first streak dilutes the cells. Go back and forth with the loop about a dozen times in the first quadrant. Be careful not to break the surface of the agar.
- For subsequent streaks, the goal is to use as much of the surface area of the plate as possible. Rotate the plate approximately 45° (so that the streaking motion is comfortable for your hand) and start the second streak. **Do not dip the loop into the starting material** (milk, yogurt, bacterial colony, or rehydrated bacteria) **again**. Go into the previous streak one or two times and then back and forth as shown about a dozen times.

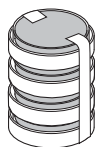
In subsequent quadrants the cells become more and more dilute, increasing the likelihood of producing single colonies. Remember a single colony arose from one cell and all the cells in the colony are genetically identical.

- Rotate the plate again and repeat streaking into the third quadrant.
- Rotate the plate again and make the final streak – **do not touch the first quadrant**.



9. Stack up your plates and tape them together. Put your group name and class period on the bottom of the stack and place upside down in a 37°C incubator for 24–48 hours.

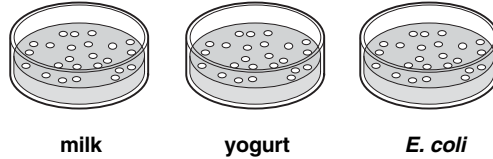
It is extremely important to follow the streak protocol to thin out the bacteria in order to have individual colonies on the plate. Otherwise the colonies may be too close together to count or pick individually.



Lesson 2: Postulate 2 Isolate Pathogen and Grow in Pure Culture

Analyze results from the previous lesson

1. Examine each of your three plates and describe what you see. Use a magnifying glass if one is available.



Are there colonies on each of the plates? Count the number of separate individual colonies and record the numbers in Table 4. The first and second quadrants may be completely covered with a lawn of bacteria.

Table 4: The number of separate individual colonies on each plate.

Yogurt	Milk	<i>E. coli</i>

2. Do all your colonies look the same? Find a colony of each type that is isolated from other colonies and circle it. Label each circle with a number on the bottom of the agar plate – not the lid. Count and record the number of different types of colonies on each plate in Table 5.

Table 5: The number of different types of colonies on each plate.

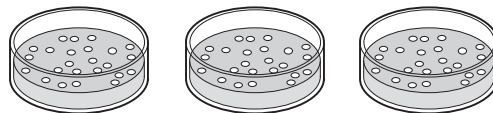
Yogurt	Milk	<i>E. coli</i>

3. Describe the appearance (morphology) of at least two of the circled colonies. Use a magnifying glass if one is available. Are the colonies large or small? Are the colonies circular or irregular? Is the edge of the colony even or irregular? Is the colony flat or raised? What is the color of the colony? Record the number of the colony, which plate it is on, and describe and draw the morphology of the colony in Table 6.

Table 6: Morphology of the bacterial colonies.

Yogurt colony #1	Yogurt colony #2
Milk colony	<i>E. coli</i> colony

4. Label some slides to correspond with your circled colonies. Use one slide for two samples as in the first lesson.

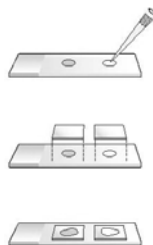


milk

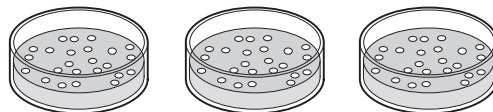
yogurt

E. coli

5. Using a new toothpick take the circled colony and put it on the slide. Add a drop of water, mix, and put a cover slip on top.



6. Repeat this procedure with the other circled colonies from the yogurt, milk, and *E. coli* plates.



milk

yogurt

E. coli

7. Examine the colony slides under the microscope at 400x magnification. Observe and draw the bacteria, their shape, and if they are linked together. Record your results in Table 7.



Table 7: Description and drawing of bacteria under a microscope.

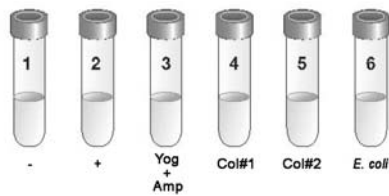
Yogurt 1	Yogurt 2
Milk	<i>E. coli</i>

8. Compare these bacteria with your description of those from the yogurt in the first lesson. Do any of the bacteria appear to be the same? If so which ones?

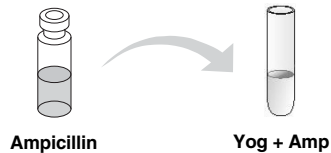
Lesson 2 continued: Postulate 3

Inoculate a healthy individual with the pure culture of suspected pathogen

9. Label the culture tubes containing milk with your initials and one of the following descriptions:
1. Negative control (milk alone)
 2. Positive control (yogurt brand)
 3. Yogurt plus ampicillin
 4. Yogurt colony #1
 5. Yogurt colony #2
 6. *E. coli*



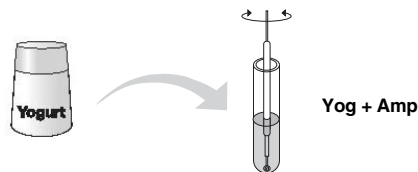
10. Add 10 μ l or one drop of the rehydrated ampicillin to tube 3 and mix well.



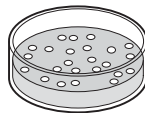
11. With a sterile loop, dip into the yogurt then swirl the loop into the milk tube 2 (positive control).



12. With a new sterile loop, dip into the yogurt then swirl the loop into the milk tube 3 (yogurt plus ampicillin).



13. Circle two different types of colonies on your yogurt plate and number them. If you have more than two different types of colonies, choose only two. If you have only one type of colony, circle two similar colonies.



yogurt

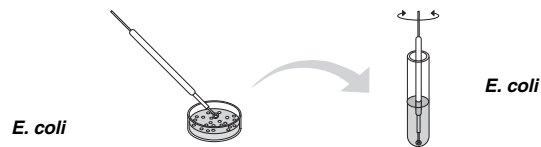
14. Using a sterile loop, transfer the single bacterial colony from yogurt colony #1 into tube 4.
Swirl the loop to mix.



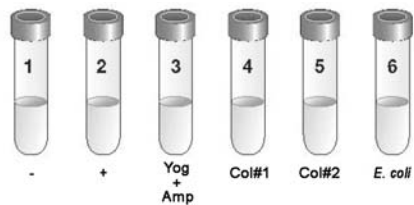
15. Using a new sterile loop, transfer the single bacterial colony from yogurt colony #2 into milk tube 5. Swirl the loop to mix.



16. Using a new sterile loop transfer an *E. coli* colony into milk tube 6 and swirl the tube.



17. Place the six tubes in a 37°C incubator for 24–48 hours.



Lesson 2 Post Lab Focus Questions

1. What could we conclude if we see more than one type of bacteria growing on an agar plate streaked with yogurt?
2. If there is more than one type of bacteria how could this affect our investigation into the "yogurtiness" disease?
3. Which of Koch's postulates are tested by adding bacteria from yogurt to milk?
4. What do you expect to see in the 6 tubes of milk after incubation?

Tube 1 – Negative control (milk alone)

Tube 2 – Positive control (yogurt brand)

Tube 3 – Yogurt plus ampicillin

Tube 4 – Yogurt colony #1

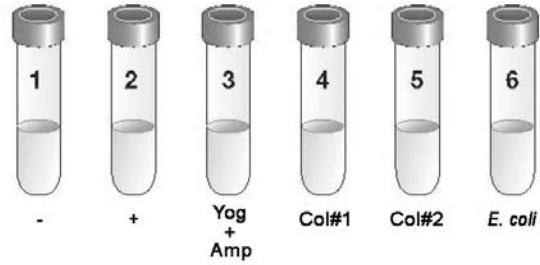
Tube 5 – Yogurt colony #2

Tube 6 – *E. coli*

- 5) Do all bacteria cause milk to turn into yogurt? Which of the controls tests for this?
- 6) Why add an antibiotic (ampicillin) in one of the tubes?

Lesson 3: Postulate 4

Isolate and identify suspected pathogen from newly diseased individual

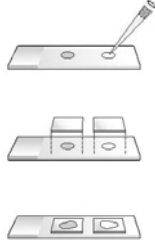


1. After the 24–48 hour incubation examine each of the 6 tubes and the original yogurt and record observations.

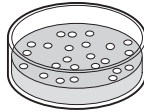
Table 8: Characteristics of milk cultures

Tube	Texture	Color	Smell	pH
Tube 1 Negative Control				
Tube 2 Positive Control				
Tube 3 Yogurt + Ampicillin				
Tube 4 First Colony				
Tube 5 Second Colony				
Tube 6 <i>E. coli</i>				
Control Yogurt (cup)				

2. Label three slides to correspond to your milk tube labels. Use one slide for two samples on the right and the left as described in the first lesson.
3. Label a fourth slide yogurt colony #1 on the right and yogurt colony #2 on the left.



4. Prepare slide samples of each milk culture for viewing under microscope as in previous lessons. For solid cultures, dip a toothpick in the culture and mix with a drop of water. For liquid cultures, add a drop to the slide. Cover with a cover slip.
5. Pick a colony from the yogurt plate similar to that used to start the yogurt cultures in tube 4 (i.e. the same colony type as yogurt colony #1). Mix the colony with a drop of water on right hand side of the appropriately numbered slide and cover with a cover slip. Repeat the procedure with yogurt colony #2 on the left of the slide.



yogurt

6. Observe slides under the microscope at 400x magnification. Describe and draw what you see in Table 9.

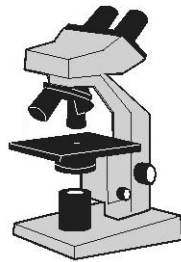


Table 9: Description of milk cultures under microscope

Tube	Description under microscope	Drawing under microscope
Tube 1 Negative control		
Tube 2 Positive control		
Tube 3 Yogurt + Ampicillin		
Tube 4 First Colony		
Tube 5 Second Colony		
Tube 6 <i>E. coli</i>		
Control yogurt (cup)		

7. Compare the newly infected cultures in tubes 4 and 5 with the pure bacteria – are they the same?

Lesson 3 Focus Questions

1. From your results, what can you conclude about what causes milk to turn into yogurt?
2. What evidence do you have to support your conclusions?
3. Can any bacteria turn milk into yogurt? What evidence do you have to support your answer?
4. Can yogurt-making bacteria be prevented from making yogurt? What evidence do you have to support your answer?
5. If you had just added yogurt to the milk and found that it made yogurt, what would that show and what would that fail to show?
6. Why is it important to inoculate milk with bacteria from a single colony rather than from multiple bacterial colonies?
7. Some bacteria will only grow when they have access to specific types of nutrients. If some bacteria in the yogurt would only grow in milk, and would not grow on agar, how would this have affected your investigation?

Appendix A

Glossary

Aerobe — Aerobes are bacteria that require oxygen for survival.

Agar — Agar is a jelly-like substance obtained from seaweed. It is made of linked sugars (a polysaccharide) and is used to make media for growing bacteria.

Ampicillin — Ampicillin is a penicillin-like bactericidal antibiotic that inhibits the synthesis of the peptidoglycan component of bacterial cells walls especially in Gram-positive bacteria but also in some Gram-negative bacteria, such as *E. coli*.

Anaerobe — Anaerobes are bacteria that do not require oxygen for survival. Anaerobes may die in the presence of oxygen.

Antibiotic — An antibiotic is a chemical that prevents or reduces the growth of bacteria or other microbes.

Anthrax — Anthrax is an often fatal disease that affects both animals and humans. *Bacillus anthracis* bacteria were identified as the cause of anthrax by Robert Koch in 1876. Anthrax is a possible agent of biowarfare or bioterrorism.

Archaea — Archaea are bacteria-like, single-celled micro organisms with no nucleus. Archaea were once thought to be a type of bacteria but are now known to be an entirely separate domain of life along with Bacteria and Eukaryota (animals, plants, fungi, and protozoans).

Bacillus — A bacillus is a rod-shaped bacteria.

Bacteria — Bacteria are single-celled, microorganisms with no nucleus. Bacteria are one of the three domains of life along with Archaea and Eukaryota (animals, plants, and fungi).

Bactericidal — An antibiotic or other agent that kills bacteria is said to be bactericidal.

Bacteriostatic — An antibiotic or other agent that prevents the growth of bacteria is said to be bacteriostatic.

Bifidobacterium bifidum — *Bifidobacterium bifidum* is a Gram-positive bacteria present in the guts of animals and humans. *Bifidobacterium bifidum* aids in digestion and thus is a "probiotic" bacteria. It is added to some varieties of yogurt.

Binary fission — Most bacteria reproduce asexually by duplicating their DNA and dividing into two equal halves.

Casein — Casein is the major protein in milk. When denatured it causes milk to solidify or curdle into yogurt or cheese. Casein is denatured by enzymes or acidic conditions but not by heat.

Clone — A clone is a group of genetically identical organisms.

Coccus — A coccus is a spherical bacteria.

Colony — A clump of genetically identical bacterial cells growing on an agar plate. Because all the cells in a single colony are genetically identical, they are called clones.

Curd — Curd is denatured milk protein (casein) which becomes solid.

Denaturation — Denaturation is the process of altering the structure of proteins by some external stress such as heat, acid, or a change in salt concentration. Casein proteins in milk are denatured to form curd.

E. coli — *Escherichia coli* is a Gram-negative facultative anaerobic bacillus bacterium. It inhabits the intestines of animals and humans and may benefit them by producing vitamin K and preventing the spread of harmful bacteria. Harmless genetically weakened forms of *E. coli*, such as the HB101 K-12 strain used in this kit, are used in many scientific applications. Normally *E. coli* is harmless but a few strains such as O157:H7 can cause disease.

Enzyme — An enzyme is a protein that catalyzes a chemical reaction. Rennin is an enzyme which causes milk proteins (casein) to coagulate (solidify) into curd.

Eukaryotes — Eukaryotes are one of the three domains of life along with Bacteria and Archaea. DNA in eukaryotic cells is contained in a special compartment called a nucleus. Eukaryotes include all animals, plants, fungi, and single-celled protozoans.

Facultative anaerobe — Facultative anaerobes are bacteria that can use oxygen but do not need it and thus can grow either in the presence or absence of oxygen.

Germ theory — Germ theory proposes that disease is caused by microbes. Disease could be transmitted from a diseased individual to a healthy individual by passage of these microbes. Prior to the acceptance of germ theory it was thought that diseases arose spontaneously. Agostino Bassi first formally stated germ theory based on his observation of disease in silkworms. Robert Koch devised Koch's postulates as a test of germ theory and demonstrated that anthrax was caused by the bacteria *Bacillus anthracis*.

Gram-positive — Bacteria whose cell walls contain only one lipid membrane surrounded by a thick layer of peptidoglycans are Gram-positive because they take up Gram stain. *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* are Gram-positive bacteria.

Gram-negative — Bacteria whose cell walls contain a second lipid membrane on the outside of a thin layer of peptidoglycans and interior lipid membrane are gram negative because they do not take up Gram stain. *E. coli* are Gram-negative bacteria.

Gram stain — Gram stain contains two dyes: crystal violet and safranin. The crystal violet is taken up by Gram-positive bacteria and will appear purple or blue. Only the safranin is taken up by Gram-negative bacteria and will appear pink or red.

Koch's postulates — A series of tests devised by Robert Koch used to assign the cause of a disease to a particular microbe.

1. The microorganism must be found in all organisms suffering from the disease, but not in healthy organisms.
2. The microorganism must be isolated from a diseased organism and grown in pure culture.
3. The cultured microorganism should cause disease when introduced into a healthy organism.
4. The microorganism must be again isolated from the inoculated, diseased experimental host and identified as identical to the original specific causative agent.

Koch, Robert — Robert Koch was a German doctor who lived from 1843 to 1910. He discovered the bacteria that caused anthrax, tuberculosis, and cholera. He also developed Koch's postulates, a series of tests used to assign the cause of disease to a particular microbe.

Lactic acid — Lactic acid ($C_3H_6O_3$) is sometimes known as milk acid. It is produced by the fermentation of lactose or other carbohydrates by lactic acid bacteria. Lactic acid causes the denaturation (or curdling) of casein into solid form. Lactic acid is a normal byproduct of metabolism and may accumulate during exercise causing temporary side pains.

Lactic acid bacteria — Bacteria that break down (ferment) sugars, such as lactose into lactic acid, are termed lactic acid bacteria. *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* are all lactic acid bacteria. Lactic acid bacteria are tolerant to acidic low pH conditions but many other bacteria are not. Thus lactic acid bacteria preserve food, such as yogurt or sauerkraut from the effects of spoilage bacteria.

LB — Luria Bertani broth (sometimes called lysogeny broth) is composed of yeast extract, tryptone, and sodium chloride and is commonly used to culture bacteria.

LBS — Luria Bertani Sugar is LB with sugar added to promote the growth of particular bacteria that would not grow as well on plain LB media.

Lactobacillus acidophilus — *Lactobacillus acidophilus* is a beneficial probiotic Gram-positive, rod-shaped lactic acid bacterium. It tolerates warm acidic conditions. It is added to some types of yogurt.

Lactobacillus bulgaricus — *Lactobacillus delbrueckii subsp. bulgaricus* is a beneficial probiotic Gram-positive, rod-shaped lactic acid bacterium. It tolerates warm acidic conditions. It is present in most types of yogurt.

Lactobacillus casei — *Lactobacillus casei* is a beneficial probiotic Gram-positive, rod-shaped lactic acid bacterium. It is added to some types of yogurt.

Lactose — Lactose ($C_{12}H_{22}O_{11}$) is often referred to as milk sugar. It is the main type of sugar in milk. Adults in many parts of the world cannot digest lactose without the aid of probiotic bacteria as are found in yogurt.

Media — Media is a substance such as an agar plate or nutrient broth that will support the growth of microbes.

Microbe — Microbes or microorganisms are single-celled organisms such as bacteria, archaea, yeast, or protozoans, usually visible only under a microscope. The term may also include viruses although they are not strictly alive.

Microbiology — Microbiology is the study of microbes.

Pasteur, Louis — Louis Pasteur was a French chemist who lived from 1822–1895. Along with Robert Koch he was one of the founders of the science of microbiology. Pasteur is famous for creating the first vaccine for rabies, a viral disease, and for devising the process of pasteurization.

Pasteurization — Pasteurization is a process used to destroy nearly all pathogenic bacteria and, most but not all of, spoilage bacteria in milk or other liquid foods. Milk is pasteurized by heating to 62.9°C for 30 min, or 71.6°C for 15 sec, and is then cooled rapidly. Pasteurization was invented in 1862 by Louis Pasteur.

Penicillin — Penicillin is a bactericidal antibiotic that inhibits the synthesis of the peptidoglycan component of bacterial cells walls especially in Gram-positive bacteria. Penicillin was discovered by Alexander Fleming in 1928 and was the first antibiotic to be used medically.

Peptidoglycan — Peptidoglycans are sugar-peptide molecules that make up part of the cell walls of bacteria. Gram-positive bacteria have a thick layer of peptidoglycan on the outside of their lipid membrane. Gram-negative bacteria have a thin layer of peptidoglycans between their two lipid membranes. Some antibiotics such as penicillin or ampicillin prevent the production of peptidoglycans. Archaea and eukaryotes do not have peptidoglycans.

Petri dish — Petri dishes are small round flat containers made of glass or plastic. They are commonly used to hold media used to culture microbes. Petri dishes were invented by microbiologist Julius Petri, an assistant to Robert Koch.

Probiotic — Probiotic bacteria aid in the digestion of foods, such as lactose in milk, and thus are added to some foods for their health benefits. The most common type of probiotics are lactic acid bacteria, such as those found in yogurt.

Pyruvic acid — Pyruvic acid ($C_3H_4O_3$) is created by the fermentation of carbohydrates. Pyruvic acid can be further fermented by some bacteria to make lactic acid.

Spontaneous generation — At one time microbes and other pests were believed to arise spontaneously from decaying matter. For instance, maggots were thought to appear spontaneously in meat. Louis Pasteur and others showed that microbes could not appear without being introduced from an external source. The idea of spontaneous generation has since been replaced by the germ theory of disease.

Streptococcus thermophilus — *Streptococcus salivarius subsp. thermophilus* is a beneficial probiotic Gram-positive spherical lactic acid bacterium. It tolerates warm acidic conditions. It is present in most types of yogurt.

Yogurt — Yogurt is a healthy food made from milk fermented by lactic acid bacteria, such as *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus salivarius subsp. thermophilus*. The bacteria make lactic acid as a byproduct which lowers the pH and curdles casein proteins in the milk. The acidic conditions help prevent growth of spoilage bacteria. Yogurt is also more digestible than milk for many people because the lactose milk sugar has been fermented into more digestible lactic acid.

Yogurtness — Yogurtness is a made up term meaning the "condition of being like yogurt".

Appendix B Instructors Answer Guide

Pre-Lab Focus Questions

1. What diseases do you know of that are caused by bacteria?

Answers may vary but some bacterial diseases are listed below:

Anthrax, bacterial meningitis, bacterial pneumonia, botulism, brucellosis, bubonic plague, chlamydia, cholera, dental caries (cavities), diphtheria, dysentery, gonorrhea, Legionnaires' disease, leprosy, Lyme disease, pertussis (whooping cough), rocky mountain spotted fever, salmonella (food poisoning), scarlet fever, shigellosis, strep throat, syphilis, toxic shock syndrome, tuberculosis, typhus

2. What diseases do you know of that are not caused by bacteria?

Answers may vary.

Some viral diseases are:

AIDS, common cold, ebola hemorrhagic fever, equine encephalitis, dengue, fifth disease, foot and mouth disease, Hanta virus hemorrhagic fever, Hepatitis A, Hepatitis B, Hepatitis C, herpes, influenza, measles (rubeola), mononucleosis, mumps, polio, rabies, roseola, rubella (german measles), SARS, smallpox, varicella (chicken pox), viral meningitis, viral pneumonia, warts, west Nile virus, yellow fever

Some environmental and autoimmune diseases are:

Alzheimer's disease, arthritis, asthma, cancer, emphysema, Parkinson's disease, lupus

Some fungal and parasitic disease are:

Athlete's foot, candidiasis, giardiasis, histoplasmosis, leishmaniasis, malaria, ringworm, river blindness, thrush, toxoplasmosis, trichinosis

Some prionic (protein caused) diseases are:

Creutzfeldt- Jacob disease, fatal insomnia, mad cow's disease,

Some genetic diseases are:

Celiac disease, down syndrome, hemophilia, muscular dystrophy, phenylketonuria, sickle-cell disease, Tay-Sachs disease

3. What characteristics allow bacteria to cause diseases?

They are small and thus can invade host organisms. They can survive outside the host at least for a brief period and thus can be transmitted. They can grow without being eliminated by the hosts immune system and create toxic waste products which harms the host.

4. How are bacterial diseases treated?

Bacterial diseases are often treated with antibiotics. Antibiotics are not effective against viruses and other non-bacterial diseases. So a common cold cannot be treated with antibiotics. Of course an individual with a bacterial disease should consult a doctor to discuss other treatment options.

5. How can the spread of bacterial diseases be prevented?

Good hygiene such as washing hands is important. It is also important to ensure that sources of water and food are clean and free of bacteria. Diseased individuals might be quarantined and prevented from having contact with healthy individuals.

6. Are all bacteria harmful? If not, describe the benefits of some bacteria.

No, most bacteria are harmless to humans. Some bacteria perform useful tasks. Bacteria in our digestive system break down indigestible foods into forms useful for humans and may even synthesize nutrients. Bacteria living in plants convert atmospheric nitrogen into a useable form (fixation). Bacteria clean up our environment by degrading toxins and dead organic matter. Bacteria are also helpful in the creation of many food stuffs, such as cheese, sauerkraut, and yogurt.

Lesson 2 Post Lab Focus Questions

1. What could we conclude if we see more than one type of bacteria growing on an agar plate streaked with yogurt?

We might conclude that one of the types of bacteria caused the disease and the other was coincidental or perhaps an "opportunistic infection." We might also conclude that both types of bacteria are necessary to cause yogurtness. We could even conclude that either type of bacteria could cause yogurtness.

2. If there is more than one type of bacteria how could this affect our investigation into the "yogurtness" disease?

We would not know for sure which type of bacteria caused the disease.

3. Which of Koch's postulates are tested by adding bacteria from yogurt to milk?

Postulate three.

4. What do you expect to see in the 6 tubes of milk after incubation?

Tube 1 – Negative control (milk alone)

The milk is probably unchanged.

Tube 2 – Positive control (yogurt, brand)

The milk probably turns into yogurt.

Tube 3 – Yogurt plus ampicillin

The milk is unchanged if the antibiotic inhibits the growth of the yogurt forming bacteria. Otherwise it will turn into yogurt.

Tube 4 – Yogurt colony #1

The milk might turn into yogurt if the first type of bacteria causes yogurt to form.

Tube 5 – Yogurt colony #2

The milk might turn into yogurt if the second type of bacteria causes yogurt to form.

Tube 6 – *E. coli*

The milk might turn into yogurt if all bacteria cause the creation of yogurt but not otherwise.

5. Do all bacteria cause milk to turn into yogurt? Which of the controls tests for this?

Tube number 6 with the *E. coli* tests if all bacteria cause yogurt to form.

6. Why are we using an antibiotic (ampicillin) in one of the tubes?

Ampicillin is an antibiotic which inhibits the growth of bacteria. If the antibiotic prevents the creation of yogurt that is additional proof that bacteria cause the production of yogurt. The addition of the antibiotic is also a test of a possible preventative measure. We did not test the antibiotic as a cure. To test a cure we would have to add ampicillin to a tube of yogurt and see if it turns back into milk. (It does not since the ampicillin cannot renature the curdled milk proteins).

Lesson 3 Focus Questions

1. From your results, what can you conclude about what causes milk to turn into yogurt?

One or more types of bacteria from yogurt causes “yogurtiness.”

2. What evidence do you have to support your conclusions?

If we have successfully followed Koch’s postulates we have: 1) isolated a possible causative agent from yogurt (the diseased individual); 2) grown that microbe in a pure culture on an agar plate; 3) reintroduced that agent into a healthy individual (milk) and seen the symptoms of the disease (the milk turns into yogurt) and; 4) again isolated the same bacteria from the newly infected individual.

3. Can any bacteria turn milk into yogurt? What evidence do you have to support your answer?

No, the *E. coli* in tube 6 should not have turned the milk into yogurt.

4. Can yogurt-making bacteria be prevented from making yogurt? What evidence do you have to support your answer?

Yes, the ampicillin should have prevented the milk in tube 6 from turning into yogurt. However we can only say this if the positive control (tube 2) does turn into yogurt.

5. If you had just added yogurt to the milk and found that it made yogurt, what would that show and what would that fail to show?

It would show that something in yogurt causes yogurt to form. It would not show what the causative agent was. It could be a bacteria, virus, fungus, prion, parasite, or even something else.

6. Why is it important to inoculate milk with bacteria from a single colony rather than from multiple bacterial colonies?

Yogurt and diseased individuals may harbor more than one kind of bacteria. We would not know what type of bacteria causes yogurt to form.

7. Some bacteria will only grow when they have access to specific types of nutrients. If some bacteria in the yogurt would only grow in milk, and would not grow on agar, how would this have affected your investigation?

The causative bacteria would not grow on the plate and thus no bacterial colony that we found would cause the disease to appear in a healthy individual. We might try the experiment again with a different, perhaps more general nutrient media in the hope of being able to grow the right bacteria. Some disease-causing bacteria, such as tuberculosis, grow poorly if at all on any artificial medium and thus it is difficult to work with them.

Appendix C

Additional Information

Microbiology

The Microbe Zoo:

<http://commtechlab.msu.edu/sites/dlc-me/zoo/>

Introduction to the Bacteria:

<http://www.ucmp.berkeley.edu/bacteria/bacteria.html>

Microbiology for the Public:

<http://www.bact.wisc.edu/GenInfo.html>

The Microbiology Network:

<http://www.microbiol.org/>

Making Yogurt

Making Yogurt Illustrated:

http://biology.clc.uc.edu/Fankhauser/Cheese/yogurt_making/YOGURT2000.htm

Making Yogurt at home:

<http://chetday.com/howtomakeyogurt.htm>

Yogurt Production:

<http://www.waksmanfoundation.org/labs/lsu/yogurt.html>

Probiotic Bacteria

Huffnagle, Gary and Wernick, Sarah, *The Probiotic Revolution*, Bantam (2008)

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