LAB TOPIC 12

Bacteriology

Laboratory Objectives

After completing this lab topic, you should be able to:

1. Describe bacterial structure: colony morphology, cell shape, growth patterns.
2. Describe the results of Gram staining and discuss the implications to cell wall chemistry.
3. Describe a scenario for succession of bacterial and fungal communities in aging milk, relating this to changes in environmental conditions such as pH and nutrient availability.
4. Practice aseptic techniques in producing bacterial streaks, smears, and lawns.
5. Describe the ecology and control of bacteria, applying these concepts to real-life situations.

For a 2-hour lab: Omit Exercise 12.2, bacterial succession in milk, and possibly the study of bacterial colony characteristics, Exercise 12.1, Lab Study A. See the Teaching Plan.

Introduction

Humans have named and categorized organisms for hundreds—perhaps even thousands—of years. *Taxonomy* is an important branch of biology that deals with naming and classifying organisms into distinct groups or categories. Much of the work of early taxonomists included recording characteristics of organisms and grouping them based on appearance, habitat, or perhaps medicinal value. As scientists began to understand the processes of genetics and evolution by natural selection, they realized the value of classifying organisms based on phylogeny, or evolutionary history. Early in the study of *systematics*, the scientific discipline that classifies organisms based on their evolutionary relationships, scientists obtained information about phylogeny from studies of development or homologous features—common features resulting from common genes. In recent years, the discipline of *molecular systematics* has become important, where scientists use biochemical evidence—studies of nucleic acids and proteins—to investigate relationships among organisms, leading to revisions in the taxonomic scheme.

Systematists continue to grapple with the complex challenge of organizing the diversity of life into categories. In the 1960s, the early system of classifying all living organisms as either plants or animals was replaced by a five-kingdom scheme that placed all prokaryotic organisms in the kingdom Monera and eukaryotic organisms in the kingdoms Protista, Fungi, Plantae, and Animalia. In the late 1970s, the microbiologist Carl Woese and his colleagues at the University of Illinois were studying DNA and ribosomal

RNA sequences in prokaryotes when they discovered a group of organisms that were dramatically different from other prokaryotes. Because of their vast differences, Woese proposed a three-domain system of classification that has become widely accepted. In the three-domain system, the three domains—Bacteria, Archaea, and Eukarya—are a taxonomic level higher and include the kingdoms, historically the broadest taxonomic category. In the three-domain system, prokaryotes are placed in one of the two domains Bacteria or Archaea. Most of the familiar organisms commonly called bacteria are placed in the domain Bacteria. Prokaryotic organisms placed in the domain Archaea share many traits with common bacteria, but they have many unique traits. The three-domain classification renders the kingdom Monera obsolete because its members are in two domains. All eukaryotes (organisms that have cells containing true nuclei) are categorized in the domain Eukarya. Three of the kingdoms of the five-kingdom scheme now placed in Eukarya—Fungi, Plantae, and Animalia—are multicellular organisms. Members of the former kingdom Protista are now in the domain Eukarya, but researchers continue to debate the number of kingdoms of protists.

All of the organisms studied in this lab topic are common bacteria, small, relatively simple, **prokaryotic,** single-celled organisms. **Prokaryotes,** from the Greek for "prenucleus," have existed on Earth longer and are more widely distributed than any other organismal group. They are found in almost every imaginable habitat: air, soil, and water, in extreme temperatures and harsh chemical environments. They can be photosynthetic (cyanobacteria, formerly called blue-green algae), using light as the source of energy, or chemosynthetic, using inorganic chemicals as the source of energy, but most are heterotrophic, absorbing nutrients from the surrounding environment. Many bacteria are beneficial to other organisms and the environment. For example, some species play an important role in decomposing dead organisms and waste products. Many bacteria are harmful to other organisms. For example, *pathogenic* bacteria cause diseases in humans and other animals.

Most bacteria have a cell wall, a complex layer outside the cell membrane. The most common component found in the cell wall of organisms in the domain Bacteria is peptidoglycan, a complex protein-carbohydrate polymer. There are no membrane-bound organelles in bacteria and the genetic material is not bound by a nuclear envelope. Bacteria do not have chromosomes as described in Lab Topic 7; their genetic material is a single circular molecule of DNA. In addition, bacteria may have smaller rings of DNA called **plasmids** (see page 248), consisting of only a few genes. They reproduce by a process called **binary fission,** in which the cell duplicates its components and divides into two cells. These cells usually become independent, but they may remain attached in linear chains or grapelike clusters. In favorable environments, individual bacterial cells rapidly proliferate, forming colonies consisting of millions of cells.

Differences in colony morphology and the shape of individual bacterial cells are important distinguishing characteristics of bacterial species. In Exercise 12.1, working independently, you will observe and describe the morphology of colonies and individual cells of several bacterial species. You will examine and describe characteristics of bacteria growing in plaque on your teeth. You and your lab partner will compare the results of all lab studies.

EXERCISE 12.1
Investigating Characteristics of Bacteria

Because of the small size and similarity of cell structure in bacteria, techniques used to identify bacteria are different from those used to identify macroscopic organisms. Staining reactions and properties of growth, nutrition, and physiology are usually used to make final identifications of species. The structure and arrangement of cells and the morphology of colonies contribute preliminary information that can help us determine the appropriate test necessary to make final identification. In this exercise, you will use the tools at hand, microscopes and unaided visual observations, to learn some characteristics of bacterial cells and colonies.

When you are working with bacteria, it is very important to practice certain **aseptic techniques** to ensure that the cultures being studied are not contaminated by organisms from the environment and that organisms are not released into the environment.

1. Wear a lab coat, a lab apron, or a clean old shirt over your clothes to lessen chances of staining or contamination accidents.
2. Wipe the lab bench with disinfectant before and after the lab activities.
3. Wash your hands before and after performing an experiment. If directed by your instructor, use disposable gloves.
4. Using the alcohol lamp or Bunsen burner, flame all nonflammable instruments used to manipulate bacteria or fungi before and after use.
5. Place swabs and toothpicks in the disposal container immediately after use. *Never place one of these used items on the lab bench!*

The bacteria used in these exercises are not pathogenic (disease-producing); nevertheless, use appropriate aseptic techniques and work with care! If a spill occurs, notify the instructor. If no instructor is available, wear disposable gloves, and wipe up the spill with paper towels. Follow this by washing the affected area with soap and water and a disinfectant. Dispose of the gloves and soiled towels in the autoclavable plastic bag provided.

Lab Study A. Colony Morphology

Materials

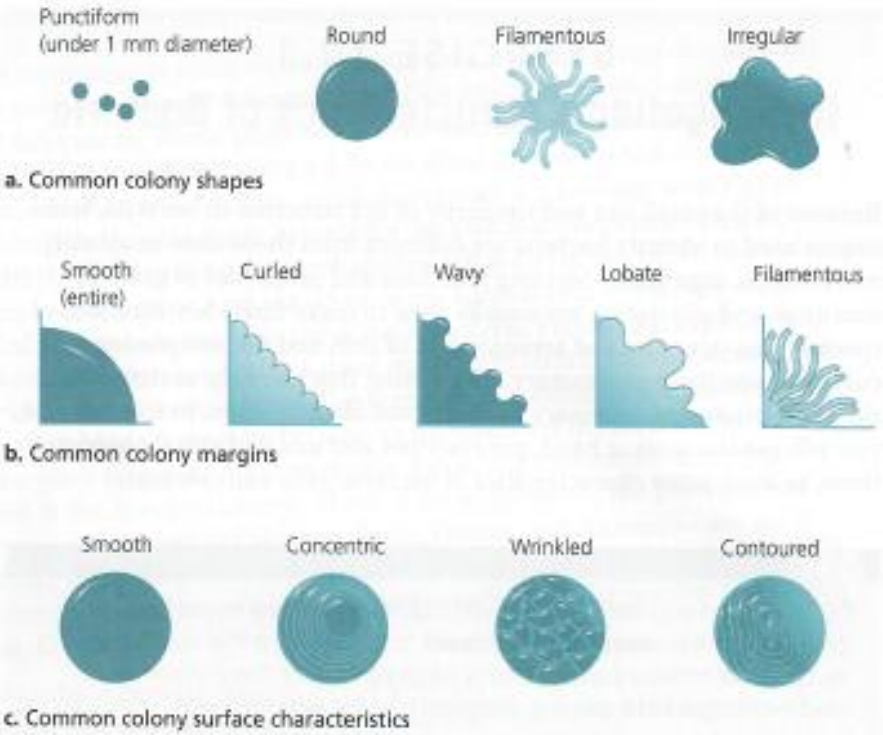
disinfectant
stereoscopic microscope
metric ruler
agar plate cultures with bacterial colonies

Provide at least six different species, six plates for every four students. Other species may be substituted. See the Preparation Guide for suggestions of bacteria. The Preparation Guide can be downloaded at masteringbiology.com in Instructor Resources, Lab Media. Seal the plates closed with Parafilm® strips. Student results will vary. Do not expect "correct" answers. The objective is to note distinguishing variations. Do not require students to learn terms used to describe bacteria. If ocular micrometers are available, have students measure colony sizes.

Introduction

A **bacterial colony** grows from a single bacterium and is composed of millions of cells. Each colony has a characteristic size, shape, consistency, texture, and color (colony morphology), all of which may be useful in preliminary species identification. Bacteriologists use specific terms to describe colony

FIGURE 12.1
Terminology used in describing bacterial colonies. (a) Common shapes, (b) margins, and (c) surface characteristics are illustrated.



characteristics. Figure 12.1 illustrates some of the terminology that may be used to describe colony morphology. Use the figure to become familiar with this terminology and describe the bacterial species provided. Occasionally, one or more **fungal colonies** will contaminate the bacterial plates. Fungi may be distinguished from bacteria by the *fuzzy* appearance of the colony (Figure 12.2). The body of a fungus is a mass of filaments called **hyphae** in a network called a **mycelium**. Learn to distinguish fungi from bacteria.

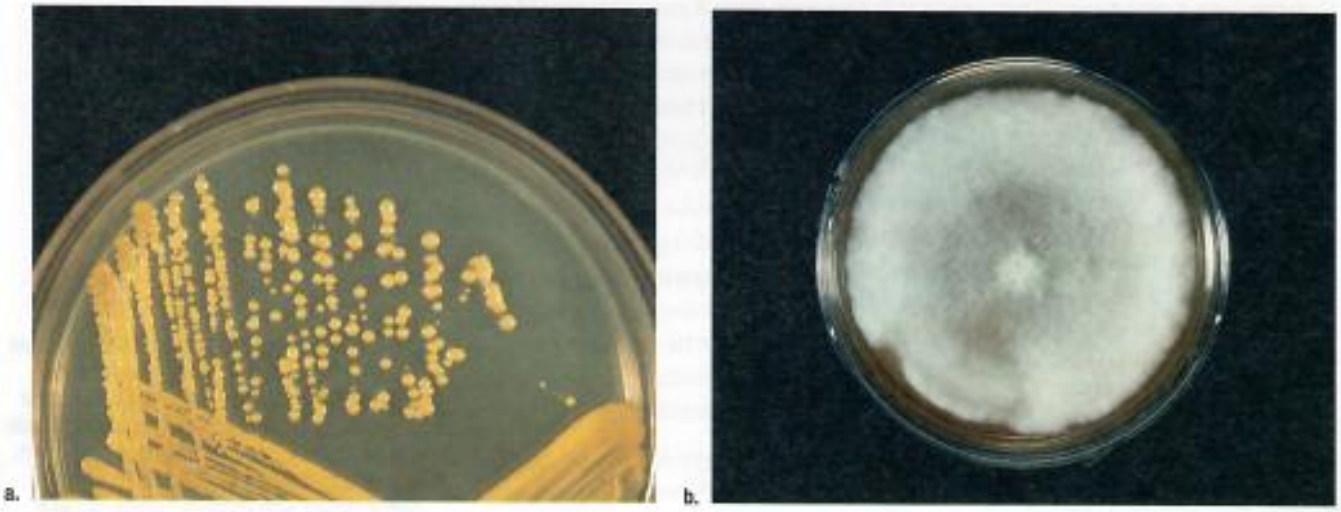


FIGURE 12.2
Distinguishing bacteria and fungi. (a) Bacterial colonies growing on a nutrient agar plate. Bacteria have been isolated using the streak technique (see Exercise 12.3, Procedure step 3). (b) Fungi growing on an agar plate. Note the filamentous fungal body, the mycelium, consisting of hyphae that give fungal colonies a fuzzy appearance.

Procedure

1. Wipe the work area with disinfectant and wash your hands.
2. Set up your stereoscopic microscope.
3. Obtain one of the bacterial plates provided. Leaving the plate closed (unless otherwise instructed), place it on the stage of the microscope.
4. Examine a typical individual, separate colony. Measure the size and note the pigmentation (none or color) of the colony, and record this information in Table 12.1 in the Results section.
5. Using the diagrams in Figure 12.1, select appropriate terms that describe the colony.
6. Record your observations in Table 12.1.
7. Sketch one colony in the margin of your lab manual, illustrating the characteristics observed.
8. Repeat steps 2 to 6 with two additional species. Your lab partner should examine three different species.
9. Observe Figure 12.2a. Describe the shape, margin, surface, and pigmentation (color) of colonies of this bacterial species.

Results

1. Complete Table 12.1 using terms from Figure 12.1 to describe the three bacterial cultures you observed.
2. Compare your observations with those of your lab partner.

Discussion

1. What are the most common colony shapes, colony margins, and colony surface characteristics found in the species observed by you and your lab partner?

2. Based on your observations, comment on the reliability of colony morphology in the identification of a given bacterial species.

Students may conclude that these criteria are not very reliable because they may find it difficult to be certain which features apply. Trained professionals and students, after practice, may be able to make initial identification based on colony morphology. However, incontrovertible identification usually depends on the results of additional tests.

TABLE 12.1 Characteristics of Bacterial Colonies

Name of Bacteria	Size	Shape	Margin	Surface	Pigmentation (none or specific color)
1.					
2.					
3.					

Lab Study B. Morphology of Individual Cells

Materials

compound microscope
prepared slides of bacillus, coccus, and spirillum bacteria
blank slide
clean toothpick
clothespin
dropper bottle of deionized (DI) water
dropper bottle of crystal violet stain
squirt bottle of DI water
alcohol lamp or Bunsen burner
staining pan

Introduction

Microscopic examination of bacterial cells reveals that most bacteria can be classified according to three basic shapes: **bacilli** (rods), **cocci** (spheres), and **spirilla** (spirals, or corkscrews). In many species, cells tend to adhere to each other and form clusters or chains of cells. In some environments, different species may associate in a complex polysaccharide matrix, creating a **community,** or assemblage of species of bacteria that adheres to a surface. These communities, called **biofilms,** are found in moist environments where nutrients are plentiful. Examples of environments that support biofilms are soils, water pipes, medical devices such as the tubes used in kidney dialysis, and the plaque found on your teeth. Anthropologists use the study of plaque on fossil teeth to determine the diets and habitats of early hominins. For example, plaque found on the teeth of a 2-million-year-old fossil of *Australopithecus sediba* disclosed that this early hominin ate fruit, leaves, wood, and bark, indicating it lived in woodland habitats. In this lab study, you will examine prepared slides of bacteria that illustrate the three basic cell shapes, and then you will examine and describe bacteria growing in your mouth.

Procedure

To save time, set this up as a demonstration or show the three types of bacteria using a video-microscopy system.

1. To become familiar with the basic shapes of bacterial cells, using the compound microscope, examine prepared slides of the three types of bacteria, and make a sketch of each shape in the space provided.

2. Your mouth is a type of ecosystem with at least 600 species of bacteria living in this warm, moist environment. Protein and carbohydrate materials from food particles accumulate at the gum line in your mouth and create an ideal environment for bacteria to grow. This mixture of materials and bacteria is a biofilm called **plaque**. To investigate the forms of bacteria found on your teeth, prepare a stained slide of plaque.
   a. Set out a clean slide.
   b. Place a drop of water on the slide. This must air-dry, so make the drop of water *small*.
   c. Using a fresh toothpick, scrape your teeth near the gum line and mix the scraping in the drop of water.
   d. Spread this plaque–water mixture into a thin film and allow it to air-dry.
   e. When the smear is dry, hold the slide with a clothespin and pass it quickly over the flame of an alcohol lamp or Bunsen burner several

times at a 45° angle. This should warm the slide but not cook the bacteria. Briefly touch the warm slide to the back of your hand. If it is too hot to touch, you are allowing it to get too warm.

Keep long hair and loose clothing away from the flame. Extinguish the flame immediately after use.

f. Place the slide on the support of a staining pan or tray and apply three or four drops of crystal violet stain to the smear (Figure 12.3).

FIGURE 12.3
Apply several drops of crystal violet stain to the slide supported in a staining pan or tray.

Crystal violet will permanently stain your clothes, and it may last several days on your hands as well. Work carefully!

g. Leave the stain on the smear for 1 minute.

h. Wash the stain off with a gentle stream of water from a squirt bottle so that the stain goes into the staining pan (Figure 12.4).

i. Blot the stained slide gently with a paper towel. Do not rub hard or you will remove the bacteria.

3. Examine the bacteria growing in the plaque on your teeth and determine bacterial forms. Use the highest magnification on your compound microscope. If you have an oil immersion lens, after focusing on the high-dry power, without changing the focus knobs, rotate the high-dry objective to the side, add a drop of immersion oil directly to the bacterial smear, and carefully rotate the oil immersion objective into place. Focus with the fine adjustment only. After observing the slide, rotate the oil immersion objective away from the slide and wipe the objective carefully, using lens paper to remove all traces of oil.

FIGURE 12.4
Gently rinse the stain into the staining pan.

Results

1. Record the individual cell shapes of bacteria present in plaque.

Cocci and bacilli are most common. Species present within a few hours after brushing include *Streptococcus mutans* (the leading cause of dental caries), *S. salivarius*, *S. sanguis*, and lactobacilli. Species in older plaque include *Corynebacterium species* (a bacillus), *Actinomyces species* (a filamentous form), and spirochetes. Students may also see yeast and large epithelial cells.

2. What shapes are absent?

Spirilli are generally less common.

3. Estimate the relative abundance of each shape.

Usually the cocci will be in greater abundance (75%) compared to bacilli (25% or less). Proportions will vary, depending on personal oral hygiene. Older plaque has more bacilli and spirilli. We once had a student with good oral hygiene who had an abundance of spirilli. He was told by his dental hygienist that this might be an indication of susceptibility to gum disease.

Discussion

1. Discuss with your lab partner information you have learned from your dentist or health class about the relationship among plaque, dental caries (cavities), and gum disease.

(You may choose to have students answer this question using online or library references.) In short, scientists have established unequivocally that bacteria cause dental caries. Bacteria convert carbohydrate to lactate, creating an acidic environment that decalcifies the tooth surface. Gum (periodontal) disease results when plaque grows under the gum rim (gingiva). Calcium is deposited in the plaque, forming tartar. The number of bacteria increases, the percentage of actinomycetes increases, and the gums become inflamed. Gums begin to bleed, they recede, and pockets form under the gums. The bone surrounding the teeth is resorbed, and the teeth loosen.

2. Suggest an explanation for differences in the proportion of each type of bacteria in the bacterial community of plaque.

Diet, time since last dental visit, and even differences in genetics will bring about differences. People taking antibiotics for other bacterial infections usually have only bacilli in their mouths.

Lab Study C. Identifying Bacteria by the Gram Stain Procedure

Materials

Bacterial colonies older than 24 hours often become gram-variable; use organisms from younger cultures for this study. You can substitute broth cultures for agar cultures. If you choose to do this, have students apply two to three loopsful of bacteria directly on the slide and spread it to make a thin film. Dry and heat the smear and proceed to stain it as directed.

compound microscope
blank slides
alcohol lamp or Bunsen burner
clean toothpicks
staining pan
cultures of *Micrococcus*, *Bacillus*, *Serratia*, and *E. coli*
dropper bottles of Gram iodine, crystal violet, safranin, DI water, 95% ethyl alcohol/acetone mixture
squirt bottle of DI water

Introduction

Gram stain is commonly used to assist in bacterial identification. This stain, first developed in 1884, separates bacteria into groups, depending on their reaction to this stain. Bacteria react by testing either **gram-positive, gram-negative,** or **gram-variable,** with the first two groups being the most common. Although the exact mechanisms are not completely understood, scientists know that the response of cells to the stain is due to differences in the complexity and chemistry of the bacterial cell wall. Recall that bacterial cell walls contain a complex polymer, **peptidoglycan**. The cell walls of gram-negative bacteria contain less peptidoglycan than the cell walls of gram-positive bacteria. In addition, the cell walls of gram-negative bacteria are more complex, containing various polysaccharides, proteins, and lipids not found in gram-positive bacteria. Gram-staining properties play an important role in bacterial classification.

Gram stain relies on the use of three stains: crystal violet (purple), Gram iodine, and safranin (pink/red). *Gram-positive* bacteria (with the thicker peptidoglycan layer) retain the crystal violet/iodine stain and appear blue/purple. *Gram-negative* bacteria lose the blue/purple stain but retain the safranin and appear pink/red (Figure 12.5).

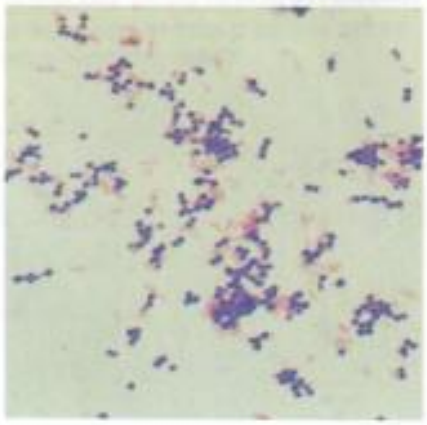


FIGURE 12.5
Gram-positive bacteria appear purple. (In this photomicrograph, these are coccus bacteria.) **Gram-negative bacteria appear pink** (the rod-shaped bacteria in this photomicrograph).