

Biology 3B Laboratory

Cultural Characteristics of Bacteria

Objectives:

- Describe bacterial structure: colony morphology, cell shape, growth patterns.
- To distinguish how various growth media will affect colony growth.
- To be able to determine bacterial species based upon macroscopic examination.
- To be able to differentiate between the three general morphological types of bacteria.

Adapted from Biology 15 Laboratory Supplemental Manual: Wrightsman, Innis and Cannon-Moloznic.

Introduction

In the old Whittaker five kingdom classification scheme; prokaryotes (“pre-nucleus”) such as bacteria are in a single kingdom, Monera. Currently, taxonomist place organisms in one of three domains. In this classification scheme, prokaryotes are placed in either the domain Bacteria or Archaea. The prokaryotes that you will examine in this laboratory are classified in the domain Bacteria and kingdom Bacteria.

Bacteria are relatively small (1 – 10 μm) and simple, single-celled organisms that has existed on earth for over 3.5 billion years. These prokaryotes are most-likely has the greatest distribution of all organisms. They are found almost everywhere from the soil to the air and even water. In these various environments, bacteria can even withstand extreme temperatures and chemically harsh environments.

Bacteria have developed numerous modes of nutrition. They can generally be classified as either an autotroph or a heterotroph. These two nutritional modes can be further subcategorized based upon whether they use light, inorganic or organic compounds. Most prokaryotes, however, are heterotrophic, where they obtain nutrients from their surrounding environment.

Part A: Cultural Characteristics on Various Growth Media

Microorganisms may show distinguishing gross morphologies when cultured on different media. This macroscopic appearance of bacteria (characteristic growth patterns which can be observed with the naked eye) is often used in their identification. In this exercise, selected strains of bacteria will be inoculated on several types of media for the purpose of observing and comparing their colonial growth characteristics.

Procedure A:



1. Carry the cultures to your desk in a careful manner so as to avoid disturbing the growth pattern in the nutrient broth. As you examine each culture, look for the specific characteristics that are described below. The following are organisms that may be included in this laboratory: *Escherichia coli*, *Streptococcus faecalis*, *Corynebacterium xerosis*, *Staphylococcus saprophyticus*, *Streptomyces albus*, *Serratia marcescens* and *Micrococcus luteus*.
2. Record your results in Table 1 for each medium.
3. Sketch each colony, illustrating the characteristics observed.

A. Nutrient agar slants - examine your cultures thoroughly and record your observations in your laboratory worksheet.

1. Observe the amount of growth - none = 0, slight = +, moderate = ++, abundant = +++.
2. Coloration - Two types of pigmentation may occur:
 - a. pigmentation occurring within the organism itself; or
 - b. water soluble pigment that diffuses into the surrounding medium.

Most organisms will lack chromogenesis (pigment production), exhibiting a white, beige, or gray growth. Pigmentation within the organism may be red, yellow, violet, or other colors (see demonstration tubes). Soluble pigments may be blue, green, yellow, brown, or other colors (see demonstration tubes). Hold the slant up to the light to examine for diffusible pigments. It may be helpful to compare the color of the agar with an uninoculated slant.

3. Opacity - Surface growth can be termed as opaque, as transparent, or as translucent (partial transparency) depending on the degree of growth (see demonstration tubes).
4. Form - The gross or macroscopic appearance of the growth from the single streak inoculation is described by comparing it to the drawings shown in Figure 1.
 - a. filiform - uniform growth along the line of inoculation.
 - b. echinulate - margins of growth have a toothed appearance.
 - c. beaded - separate or semiconfluent colonies.
 - d. effuse - growth is thin, veil-like, unusually spreading.
 - e. arborescent - branched, tree-like growth.
 - f. rhizoid - root-like appearance.

B. Nutrient broth tubes - examine your cultures thoroughly and record your observations in your laboratory notebook.

1. Surface - Refer to Figure 2 on the following sheets. A pellicle type of surface differs from the membranous type in that the latter is much thinner. A flocculent surface is made up of floating adherent masses of bacteria.
2. Subsurface - Below the surface of the broth, the medium may be described as one of the following:
 - a. turbid - the medium is cloudy.
 - b. granular - the medium has small particles visible in suspension.
 - c. flocculent - the medium has small masses visible in it.
 - d. flaky - the medium has large particles visible in suspension.

C. Nutrient agar (NA) plate cultures - Colonies on the NA plates should be studied with respect to their color and opacity (see NA slants above), and for their configuration, elevation, and margin (see Figure 3 on the following sheets). Examine several isolated colonies with a magnifying lens or with a dissecting microscope if you need. **Do not open the plates.**

D. Motility medium (May not be available) – This test is used to demonstrate the ability of bacteria to move and is the presumptive test for the presence of flagella. To be considered motile, the growth of the bacteria must extend outward from the line of inoculation in all directions. It may be helpful to compare your inoculated tube with an uninoculated one.

Part B: Microscopic examination of various bacteria

The type of cell wall that a bacterium has can be determined by utilizing various staining techniques. One such technique is called a Gram stain. This technique involves a series of stains (crystal violet, Gram iodine and safranin) that are applied to bacterial cells on a slide. Gram positive bacteria have a thicker peptidoglycan layer, thus these bacteria are able to retain the crystal violet/iodine color and appear purple. Gram-negative bacteria have less peptidoglycan than gram-positive bacteria. As a result, gram-negative bacteria will lose the purple color but retain the safranin and appear pink/red instead. This is due to their cell wall having less peptidoglycan and being more complex with various proteins, polysaccharides and lipids.

The cell wall affords some protection to bacteria. In addition to the cell wall, many bacteria can secrete a gelatinous capsule around themselves. The capsule may allow the bacteria to adhere to substances, but may also decrease the host cell's ability to destroy them. The lipopolysaccharide layer of gram-negative bacteria also increases protection by decreasing the ability of the host to rid itself of these bacteria. This outermost layer may even be toxic. Of greater importance is this layer's ability to make it resistant to antibiotics by preventing drug entry into the organism.

In this section, you will examine several fixed slides of bacteria, some of which cause human diseases of great importance.

Procedure B

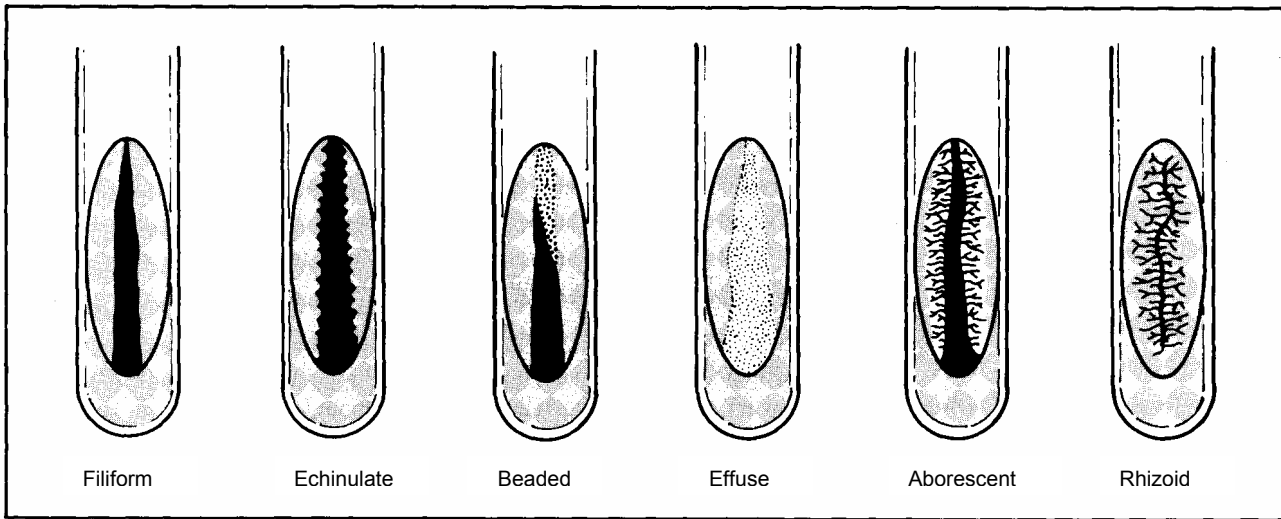
1. Obtain a slide of a typical bacillus, coccus and spirillum bacteria. Make clear sketches for each.
2. Make clear sketches, determine the size and the disease, if any, for each of the following:
 - a. *Salmonella typhi*
 - b. *Bacillus anthracis*
 - c. *Staphylococcus aureus*
 - d. *Streptococcus lactis*
 - e. *Mycobacterium tuberculosis*
 - f. *Neisseria gonorrhoeae*
 - g. *Klebsiella pneumoniae*



After you are finished with your microscope, DO NOT FORGET TO WIPE THE OIL OFF OF THE OBJECTIVE LENS AND SLIDE!!!



Figure 1. Agar Slant Growth Patterns



**Figure 2.
Surface Growth Patterns
in Nutrient Broth**

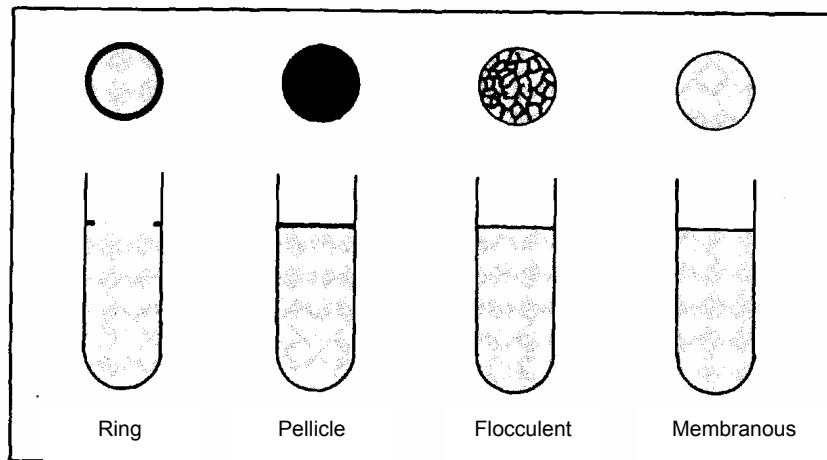
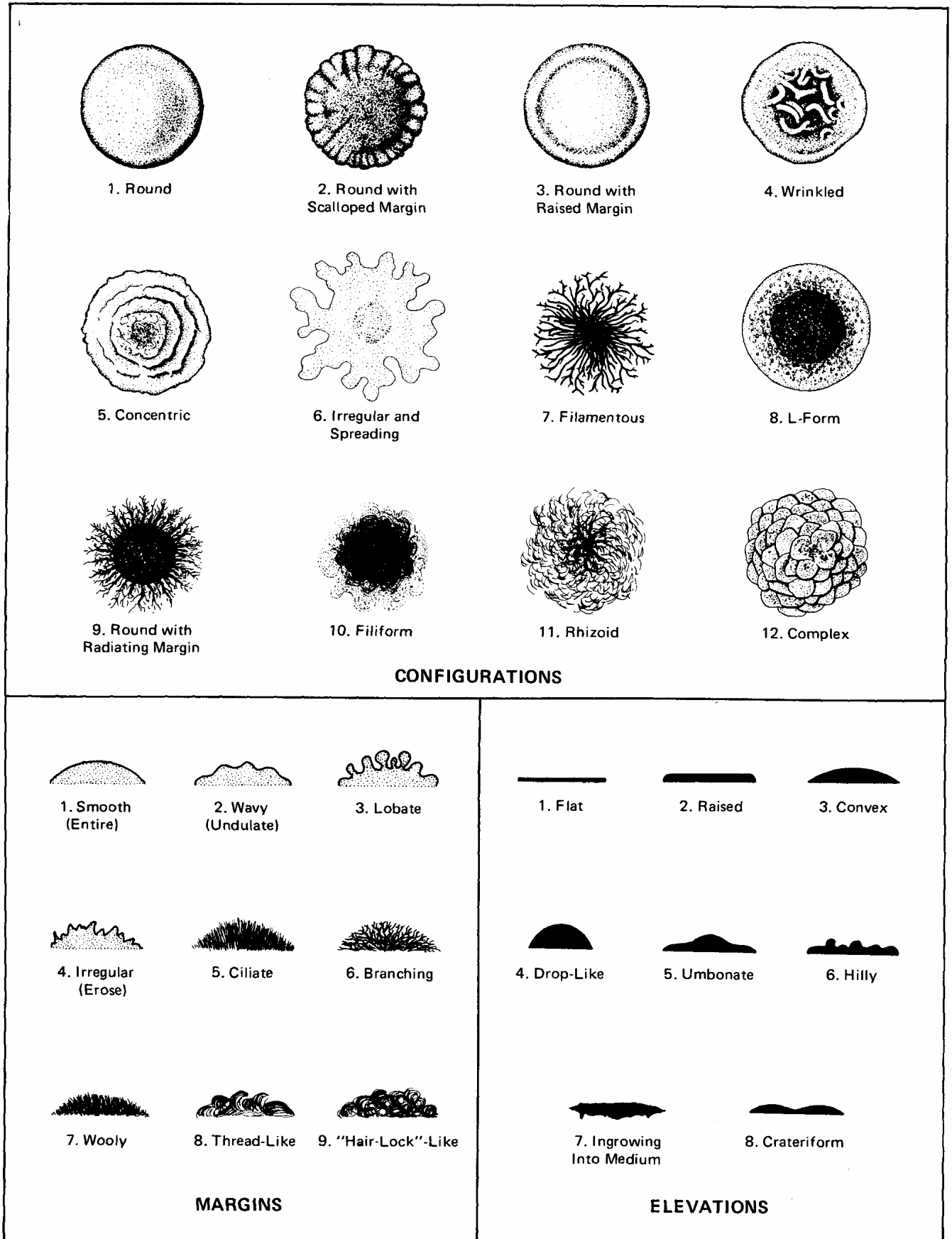


Figure 3. Colony Characteristics on Agar Plates



Biology 3B Laboratory Worksheet
Cultural Characteristics of Bacteria

Name: _____

Part A: Cultural Characteristics of Bacteria

1. What are the most common:
 - a. Colony shapes: _____
 - b. Colony margins: _____
 - c. Colony surface characteristics: _____

2. Based upon ***your own*** observations, comment on the reliability of colony morphologies in the identification of a given bacterial species.

Part B: Microscopic examination of bacteria

3. Draw your bacterial species below (include magnification). Indicate the disease next to the species name, if there is one.

