A Laboratory Manual

MICROBIOLOGY

Customized for
Morton University

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Microbial Growth 2

Microorganisms are extraordinarily diverse, and every species demonstrates a unique combination of characteristics, some of which can be easily observed. In this section we illustrate some of those characteristics and factors that affect them.

You will begin this section with an exercise intended to sensitize you to the diversity of microbial populations living all around us. Allowing for variables, such as the growth medium and incubation conditions, much can be determined about an organism by simply looking at the colonies it produces, or its appearance on slants or in broths. Distinguishing growth patterns on or in different media is an important skill—one that you can use as you progress through the semester. Note the growth characteristics of all the organisms provided for your laboratory exercises, and jot them down or even sketch them. When the time comes to identify your unknown species, you may find your records very useful.

Next, you will examine microbial nutritional diversity by growing bacteria on media with varying amounts of carbon and nitrogen resources. Following that, you will look at some environmental factors affecting microbial growth, such as oxygen, temperature, pH, and osmotic pressure. Finally, you will examine some physical and chemical microbial control agents and systems, that is, ways in which humans can control bacterial growth.

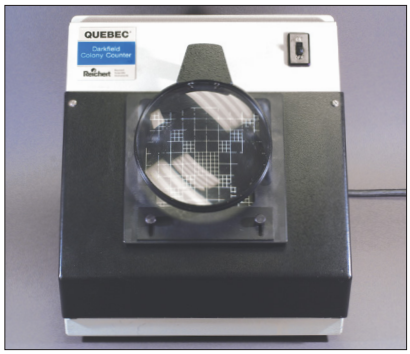
EXERCISE 2.1

Colony Morphology

Theory

When a single bacterial cell is deposited on an appropriate solid nutrient medium, it begins to divide. One cell makes two, two make four, four make eight . . . one million make two million, and so on. Eventually a visible mass of cells—a **colony**—appears where the original cell was deposited. Color, size, shape, and texture of microbial growth are determined by the genetic makeup of the organism (in many cases by yet unknown mechanisms), but are also greatly influenced by environmental factors, including nutrient availability, temperature, and incubation time.

Colony morphological characteristics may be viewed with the naked eye, a hand lens, a stereo (dissecting) microscope, or a colony counter (Fig. 2.3). The seven basic categories include colony size, shape, margin (edge), surface, elevation, texture, and optical properties (Fig. 2.4).



2.3 Colony Counter ■ Subtle differences in colony shape and size can best be viewed with magnification, such as is provided by a colony counter. The transmitted light and magnifying glass allow observation of greater detail; however, colony color and many other features are best determined with reflected light. The grid in the background is a counting aid; each big square is 1 square centimeter.

1. *Size* is simply a measurement of the colony's dimensions—the diameter if circular or length and width if shaped otherwise.
2. *Shape* may be described as **round (circular)**, **irregular**, or **punctiform** (tiny, pinpoint).
3. The *margin* may be **entire** (**smooth**, with no irregularities), **undulate** (wavy), **lobate** (lobed), **filamentous** (unbranched strands), or **rhizoid** (branched like roots).
4. The *surface* may be **smooth, rough, wrinkled** (**rugose**), **shiny**, or **dull.**
5. The *texture* may be **moist, mucoid** (sticky), **butyrous** (buttery), or **dry.**
6. *Elevations* include **flat, raised, convex, pulvinate** (very convex), and **umbonate** (raised in the center).
7. Other useful features include **color** and optical properties such as **opaque** (you can't see through it) and **translucent** (light passes through).

Features such as colony shape, margin, surface, texture (shiny or dull), and color are best viewed by observing from above while holding the plate level with the lid off (if it is safe to do so), but rocking it back and forth slightly so reflected light hits it at different angles. If allowed to do so, you may also check texture by touching the growth with an inoculating loop or wooden stick. Be sure to flame the loop afterward or dispose of the wooden stick properly.

Elevations are best viewed with the plate tilted slightly at eye level. Opacity and translucence are best viewed by placing the plate on a colony counter or holding it (lid on) so it is illuminated from behind (transmitted light). Colony dimensions are best measured from the plate's base rather than through the lid.

When reporting colony morphology, it is important to include the medium and the incubation time and temperature, all of which can affect a colony's appearance.

Application

Recognizing different bacterial growth morphologies on agar plates is a useful step in the identification process. It is often the first indication that one organism is different from another. Once purity of a colony has been confirmed by an appropriate staining procedure (this is not always done), cells can be transferred to a sterile medium, grown, and maintained as a pure culture, which then acts as a source of that microbe for identification or other purposes.

Procedure 1

Sampling of Bacterial Colony Features

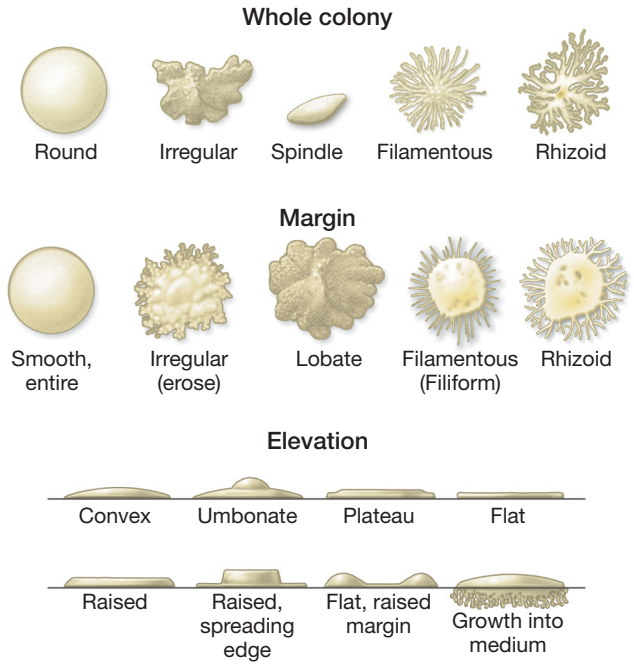
Materials

- ❑ Lab coat
- ❑ Disposable gloves
- ❑ Chemical eye protection
- ❑ Per Student Group
- ❑ (Optional) colony counter, stereo (dissecting) microscope, or hand lens
- ❑ Metric ruler
- ❑ Plates from Exercise 2-1
- ❑ (Optional) tryptic soy agar or brain-heart infusion agar streak plate cultures of any of the following:
  - ❑ Bacillus subtilis
  - ❑ Corynebacterium xerosis

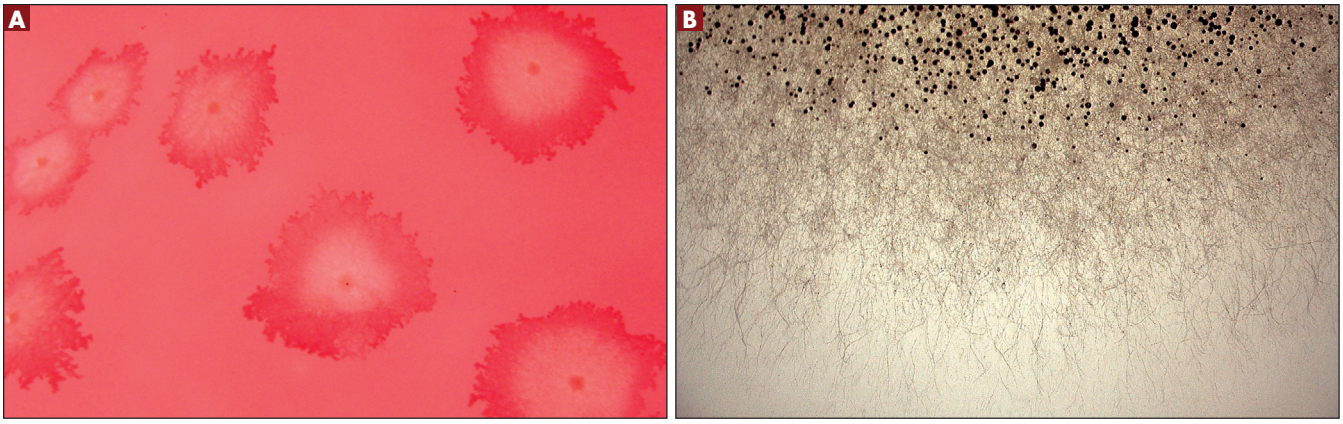
Today you will be viewing colony characteristics on the plates saved from Exercise 2-1 and (if available) prepared streak plates provided by your instructor. Figures 2.4 through 2.30 show a variety of bacterial colony forms and characteristics. Where applicable, contrasting environmental factors are indicated.

1 Working with your group, use the terms in Figure 2.4 and in the text to describe some representative colonies on your plates from Exercise 2-1 (if not already described) and the pure cultures supplied. Figures 2.5 through 2.30 may also be useful. Measure colony diameters (in mm) with a ruler and include them with your descriptions in the table on the data sheet, page 77. If you see a distinctive feature that has not been given a name, make up one! Just make it descriptive and easily understood by others. That's what the early microbiologists did to compile the list you have been given. (***Note:*** Remember that many microorganisms are opportunistic pathogens, so be sure to handle the plates carefully. **Do not open plates with BSL-2 organisms on them or those containing fuzzy growth**, because a fuzzy appearance suggests fungal growth containing spores that can spread easily and contaminate the laboratory and other cultures. If you are in doubt, check with your instructor.)

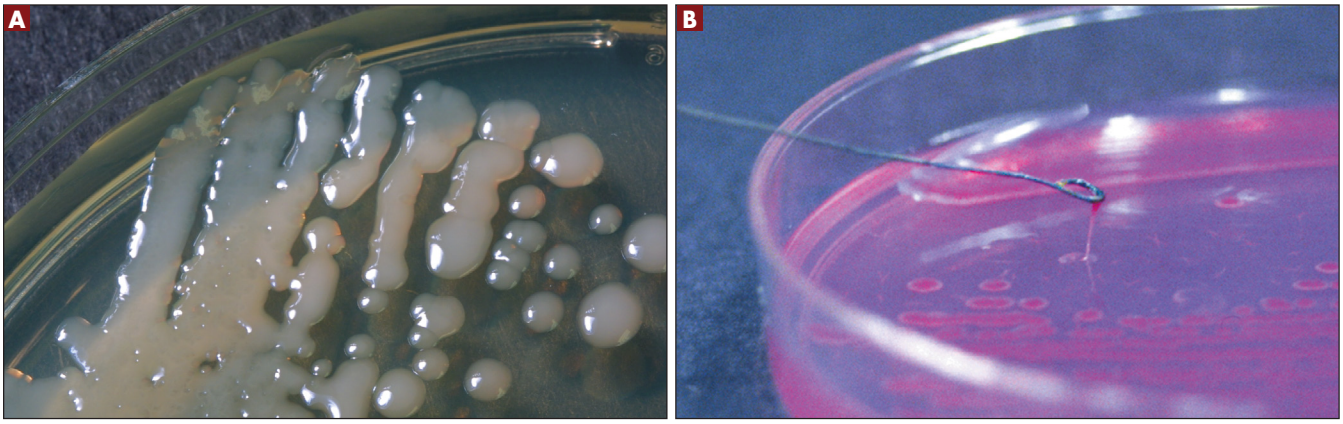
2 Unless you have been instructed to save today's cultures for future exercises (such as Exercise 2-3), discard all plates in an appropriate autoclave container.



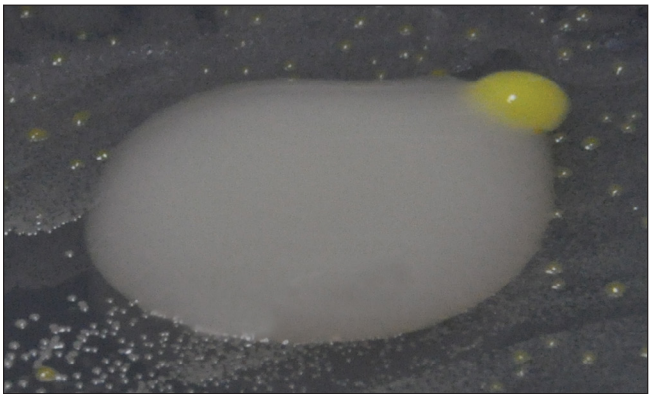
2.4 A Sampling of Bacterial Colony Features ■ These terms are used to describe colony morphology. Descriptions also should include color, size, surface characteristics, texture, and optical properties (opaque or translucent). See the text for details.



2.18 Rhizoid Margins ■ (**A**) These irregular colonies of Clostridium sporogenes were grown anaerobically on sheep blood agar and are viewed through a stereo microscope. They have a raised center and a flat, spreading edge of branched, tangled filaments (reminiscent of the mythological creature Medusa, who had snakes for hair!). They vary in size from 2 mm to 6 mm. C. sporogenes is found in soils worldwide. (**B**) Fungi (other than yeast) are naturally filamentous and sometimes the filaments are branched, as in this lab contaminant (probably Aspergillus niger). It takes magnification to see that the margin is rhizoid and not simply filamentous. The black spheres are asexual spores called conidia.



2.19 Mucoid Colonies ■ (**A**) These Klebsiella pneumoniae colonies grown on nutrient agar are mucoid, raised, and shiny. While it is a normal inhabitant of the human intestinal tract, it is associated with community-acquired pneumonia and nosocomial urinary tract infections. (**B**) Pseudomonas aeruginosa grown on Endo agar illustrates a mucoid texture. P. aeruginosa is found in soil and water and can cause infections in burn patients.



2.20 Butyrous Colony ■ This unidentified 12 mm colony was found on a glycerol yeast extract plate inoculated with a diluted soil sample. Butyrous (butyrum means "buttery") colonies have the consistency of melted butter. This colony was almost liquid in texture, something that is demonstrated by its contact with the yellow colony to its right.

2.21 Granular Colony ■ These colonies of Streptomyces griseus grown on brain-heart infusion agar are circular, entire, and granular with a ridged surface. At a later stage of development, they produce yellow reproductive spores. Growth of streptomycetes is associated with an "earthy" smell. This one plate fragranced the entire incubator!

Chapter 2 Review

Name _____ Date _____ Section _____

1 In the table below, use the terms provided in Figure 2.4 and the text to describe and carefully sketch representative colonies on the plates you examined. Draw separate sketches for the colonies as seen from above and in elevation (from the side). Plates from Exercise 2-1 can also be used, if not already examined for colony morphology. Use a colony counter for magnification if necessary. Measure colony diameters, and include them with your descriptions along with incubation times and temperatures, and culture sources.

Organism/Plate	Colony Description and Sketch