Module 2 Introduction to Culture Techniques

LEARNING OUTCOMES

1. Identify and compare forms of solid and liquid media.
2. Define colony and colony forming unit.
3. Describe growth patterns in liquid and solid media using appropriate terminology.

Culture Media

In nature, microorganisms exist as mixed populations of distinct species of bacteria, fungi, and even viruses. To study, characterize, and identify microorganisms, it is often necessary to cultivate them at a preferred temperature using a nutrient base called a *medium* (plural, *media*). Two commonly used physical forms of growth media are in the form of liquid broth and semisolid agar.

Broth can be used to determine growth patterns and are the medium of choice for growing large quantities of organisms. Semisolid agar is essentially broth with the addition of a polysaccharide thickening agent derived from red algae called agarose. Agarose is an effective solidifying agent because it withstands the high temperature needed for sterilization of the medium and is not broken down by bacteria. Forms of semisolid media include agar plates, agar slants, and agar deeps (Figure 2.1). Agar plates are made by pouring melted media into a Petri dish and allowing it to cool. Plates can be used to separate mixtures of bacteria and to observe colony characteristics of different species of bacteria. To make agar slants or agar deeps, melted agar is poured into a test tube and then allowed to solidify on an angle (agar slant), or vertically (agar deep). Agar slants are commonly used to generate stocks of bacteria, while deeps are often used to observe bacterial motility.

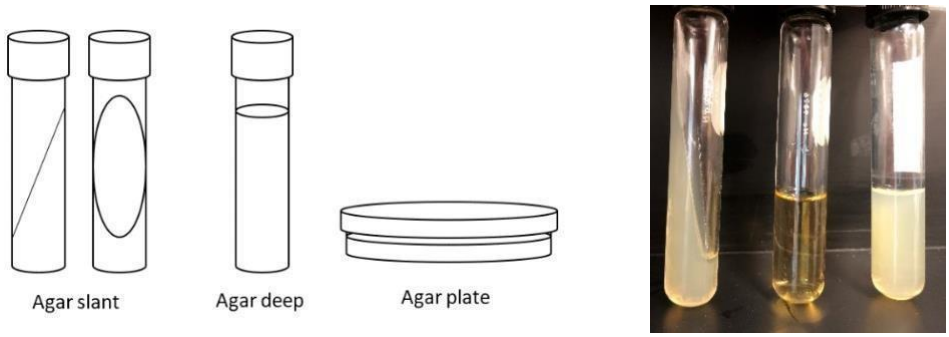

Figure 2.1: Forms of solid media (left); nutrient agar slant, broth, and deep (right).

Although individual bacteria cells are too small to be viewed with the naked eye, microorganisms form certain patterns of growth that are easily observed when they grow on or in media. These distinguishing characteristics help us to differentiate and identify organisms that are present. Figure 2.2 illustrates common growth patterns in liquid and on solid media.

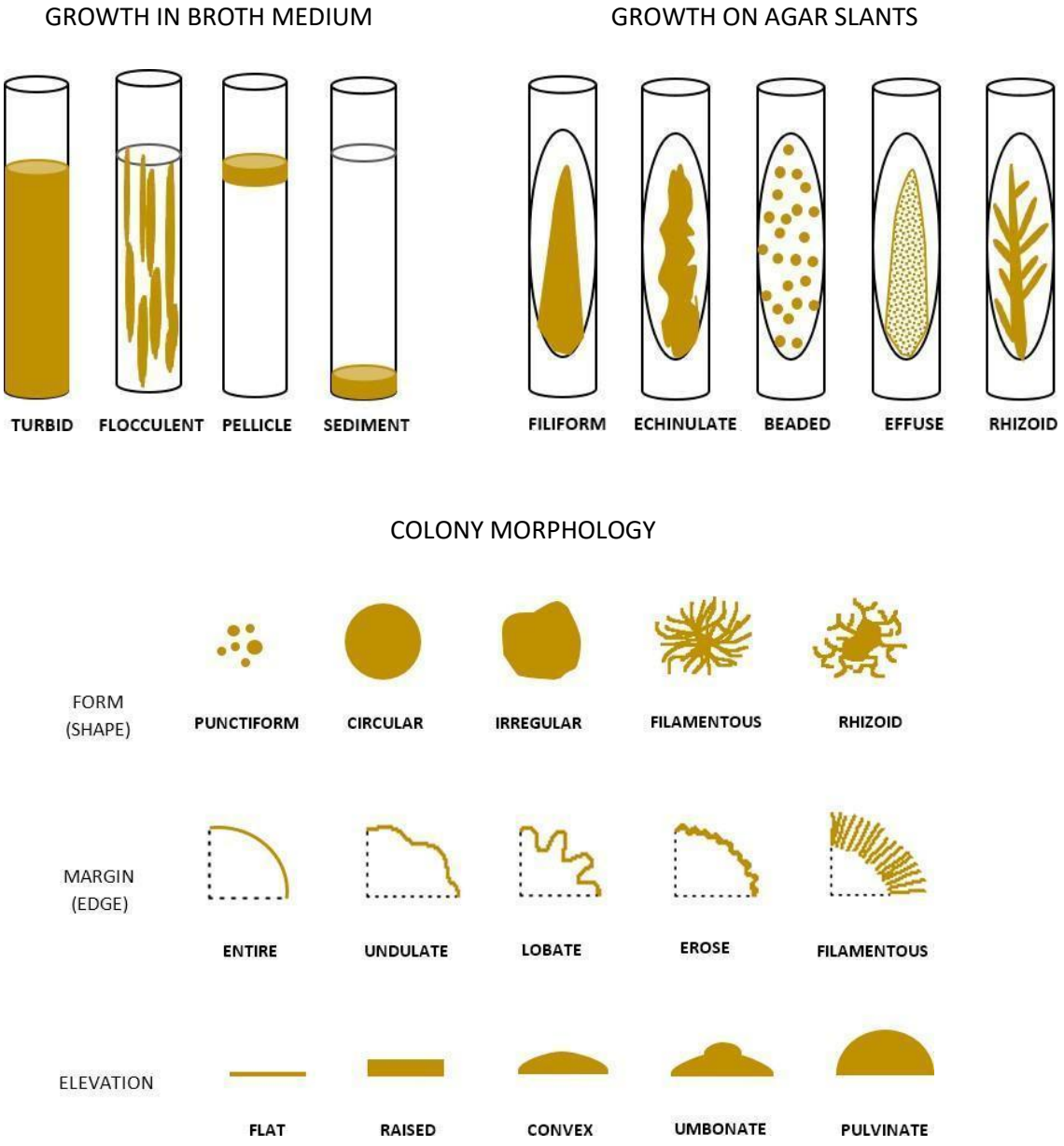

Figure 2.2: Patterns of growth in liquid and semi-solid media.

Growth Patterns in Liquid Media

Growth patterns in broth should be observed and evaluated without disrupting or shaking the tube. Many bacteria exhibit uniform *turbidity* (cloudiness) throughout the broth while others form a *sediment* at the bottom. In the latter case, the broth may be slightly turbid or clear. Some bacteria grow as a *pellicle* or film on the surface of the broth or form a ring around it. Heavy pellicles may sink a bit during incubation and appear just below the surface of the broth. Bacteria may also exhibit *flocculence,* or discrete clusters of growth, which are suspended in clear broth throughout the tube (Figure 2.3).

It is important to note that trypticase soy broth is not a reduced medium, meaning that oxygen has not been removed from the broth by chemical or other means. Since oxygen is present throughout the tube, growth patterns in non-reduced broth are not necessarily indicative of an organism's preference for oxygen. Later in the course, we will test bacteria for aerotolerance using several methods, including the use of reduced broth media.

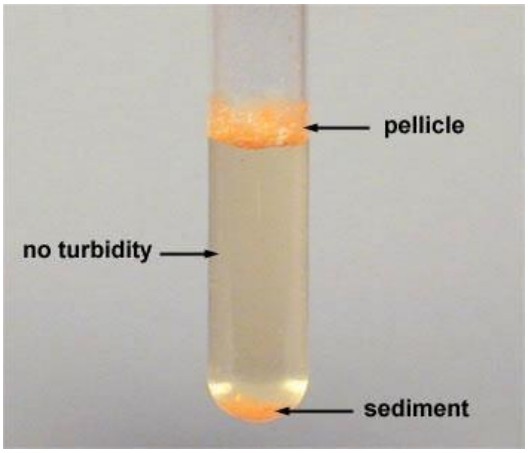

Figure 2.3. Patterns of bacterial growth in liquid media.

Growth Patterns on Semi-Solid Media

Bacteria grow on an agar surface as visible masses of cells called *colonies*. Each colony is composed of thousands to millions of cells that originated from a single bacterium or small group of bacterial cells called a *colony forming unit (CFU).* Colonies have distinguishing features including the pigment or color, the overall shape or form, the elevation of colonies when viewed from the side, the *margin* or edge, and surface texture. Evaluation of colony morphology is often subjective, which is why more than one descriptor is used (Figure 2.4).

Similar growth characteristics to those observed on plates can be seen on slants. There are additional slant characteristics that experienced microbiologists use to evaluate growth. Those observed on a slant.

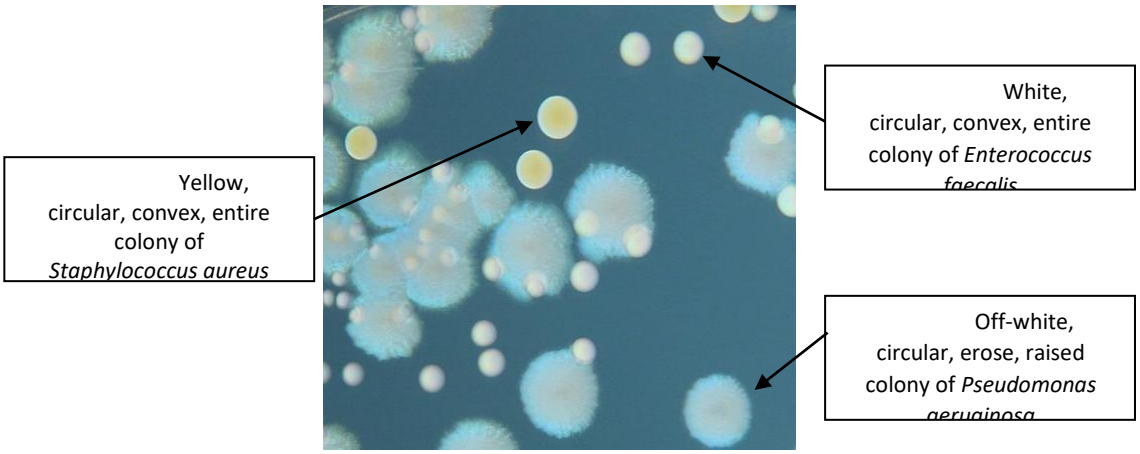

Figure 2.4: Three different colony types growing on trypticase soy agar. Colony descriptors include those for form (overall shape), elevation, and margin (edge). Colony pigment and surface texture (e.g., smooth, shiny, rough) is often evaluated as well