Al-Anbar University

College of Sciences

Biology department

Subject name: Microbial Identification

Educational level: Master

Lecture title: Examination of Growth in Broth

Subject teacher

Assistant prof. Dr. Jamal A. Rahman

EXAMINATION OF GROWTH IN BROTH

Chopped meat medium contains beef heart, peptone, dextrose, and particles of cooked meat. This liquid medium is used when examining clinical samples for anaerobic bacteria, such as *Clostridium botulinum* and *Clostridium perfringens* (Figure 4.46).

Thioglycollate broth contains casitone, yeast extract, and dextrose. It also contains thioglycollate, cystine, and agar, which lower the oxygen content of the liquid medium, making it useful for detecting anaerobic and facultatively anaerobic bacteria in clinical samples (Figure 4.47). It is also used to detect bacteria in normally sterile materials, such as pharmaceutical products.

Tryptic soy broth is a rich nutritional medium, containing tryptone, soytone, and dextrose. It is used as a general-purpose liquid medium for culturing and maintaining a wide variety of bacteria (Figure 4.48). In addition, its specific applications include the detection of bacteria in live vaccines and cosmetics.

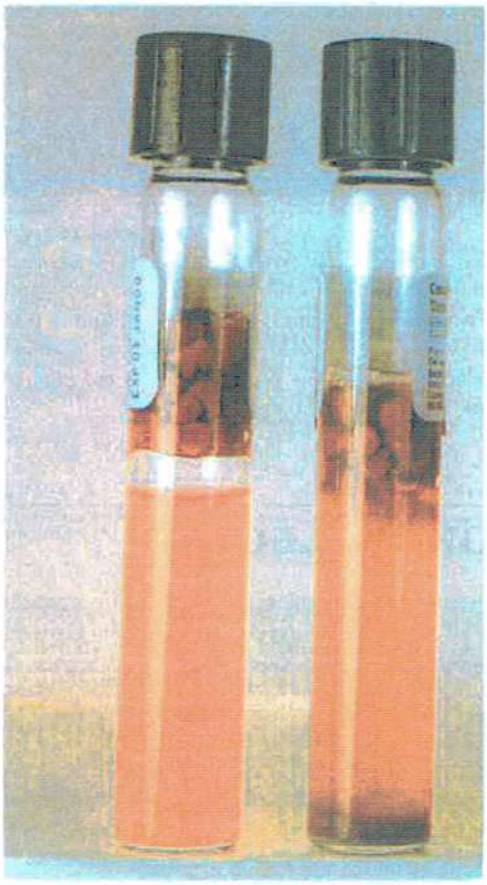

FIGURE 4.46 Chopped meat medium tubes in which bacterial growth and gas production are evident. Gas produced by the cultures has lifted the cooked meat particles to the surface.

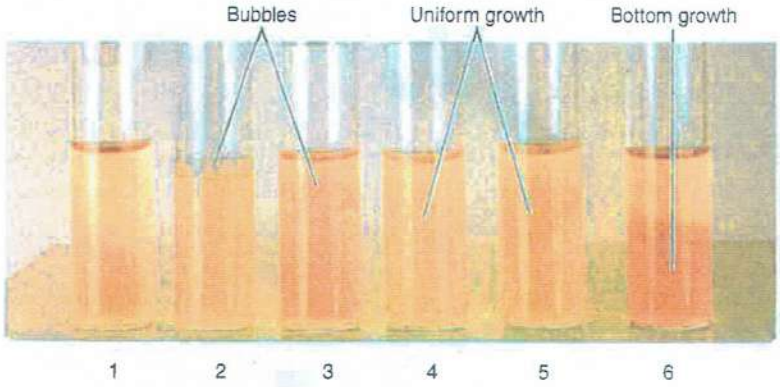

FIGURE 4.47 Bacterial growth patterns in thioglycollate broth tubes. Bubbles (tubes 2 and 3) are indicative of gas-producing bacteria; uniform growth (tubes 4 and 5) is indicative of facultatively anaerobic bacteria; and bottom growth (tube 6) is indicative of anaerobic bacteria. Tube 1 was left uninoculated.

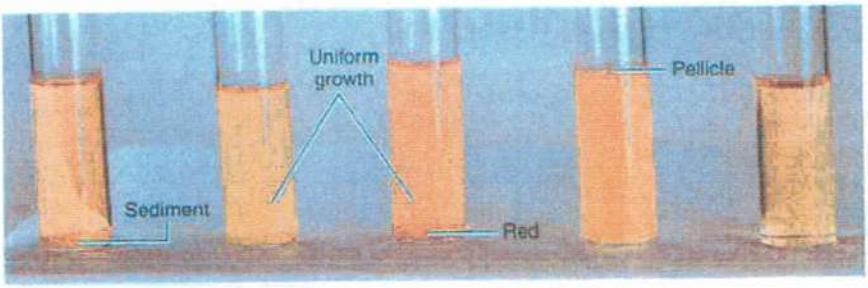

FIGURE 4.48 Tryptic soy broth tubes inoculated with (from left to right) *Bacillus cereus, Escherichia coli, Serratia marcescens, Pseudomonas aeruginosa,* and a tube left uninoculated. Growth on the bottom (sediment) is typical for *B. cereus* in broth. Facultative anaerobes, such as *E. coli* and *S. marcescens,* typically show uniform growth in broth, while aerobes, such as *P. aeruginosa,* typically show surface (pellicle) growth. The production of a red pigment, a characteristic of *S. marcescens,* is evident in the middle tube.

COLONY SELECTION

Environmental and clinical samples typically contain a number of different bacterial types. When grown on an agar medium, each type will form colonies with a unique appearance. In some cases, colonies must be examined carefully to detect differences, a process aided by the magnification of a **colony counter** (Figure 4.49) or **dissecting microscope** (Figure 4.50). Colonies may differ in shape, margin, elevation, size, texture, appearance, pigmentation, and optical properties (Figure 4.51). These colony characteristics are used for selecting specific bacterial types for isolation.

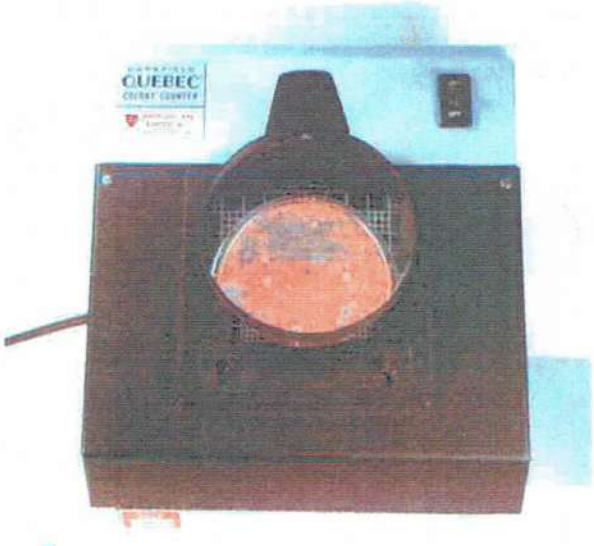

FIGURE 4.49 A colony counter. The magnification it provides can aid in the selection of colonies from agar plates.

FIGURE 4.50 A dissecting microscope, which enables careful examination for the selection of colonies on agar plates.

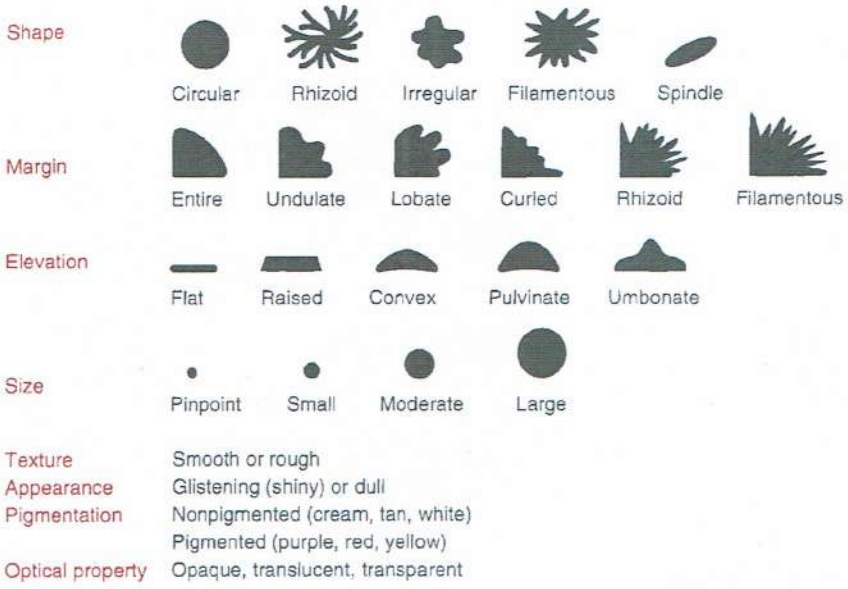

FIGURE 4.51 Aspects of bacterial colony morphology. The characteristics of bacterial colonies are described using these terms.

Lab Procedure

ISOLATION BY THE STREAK-PLATE METHOD

Purpose and Procedure Summary

Once different bacterial types have been identified based on colony characteristics, they can be transferred to a separate agar medium using a technique called the **streak-plate method.** This method is used for two reasons: (1) to confirm that only one bacterial species is transferred to a medium, thus creating a **pure culture,** and (2) to assess the specific characteristics of isolated colonies. The streak-plate method is summarized in Figure 4.52.

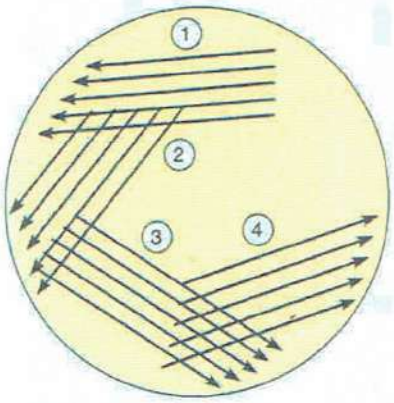

FIGURE 4.52 The streak-plate method. Cells from a selected colony are picked up on the end of a sterile loop and transferred to area 1 by rubbing the loop across the surface of the agar, creating visible "streaks." After streaking area 1, the loop is sterilized and then rubbed across the edge of area 1 to make area 2. This procedure is repeated for areas 3 and 4. Cells deposited in area 1 are now sufficiently spread out to allow for the development of isolated colonies.

Tips for Success

- *Sterilize* the loop prior to each step. Be aware that the loop is not sterilized by simply waving it over a Bunsen burner flame. The end of the wire and the entire loop must glow red in the flame, as seen in Figure 4.53, to ensure sterility.
- Transfer only a *pin-head sized* amount of growth from a single colony. If too many cells are transferred, they cannot be spread out sufficiently to ensure isolated colonies.
- *Cover the plate* after each set of streaks. Leaving a plate open too long can result in contamination such as that depicted in Figure 4.54.
- Spread cells over the *entire* plate.

Expected Results

A streak plate of *Chromobacterium violaceum* is depicted in Figure 4.55. Notice that colonies are well separated and that only one bacterial type, with distinct characteristics, is present. These colonies are circular, entire, convex, smooth, shiny, moderate, purple, and opaque.

Streak plates of other common bacteria are presented in Figures 4.56 through 4.69. Notice that some bacteria can have different appearances, depending on the medium on which they are grown. For example, *Pseudomonas aeruginosa* looks different on nutrient agar (Figure 4.63) than on either blood agar (Figure 4.64) or Mueller Hinton agar (Figure 4.65).

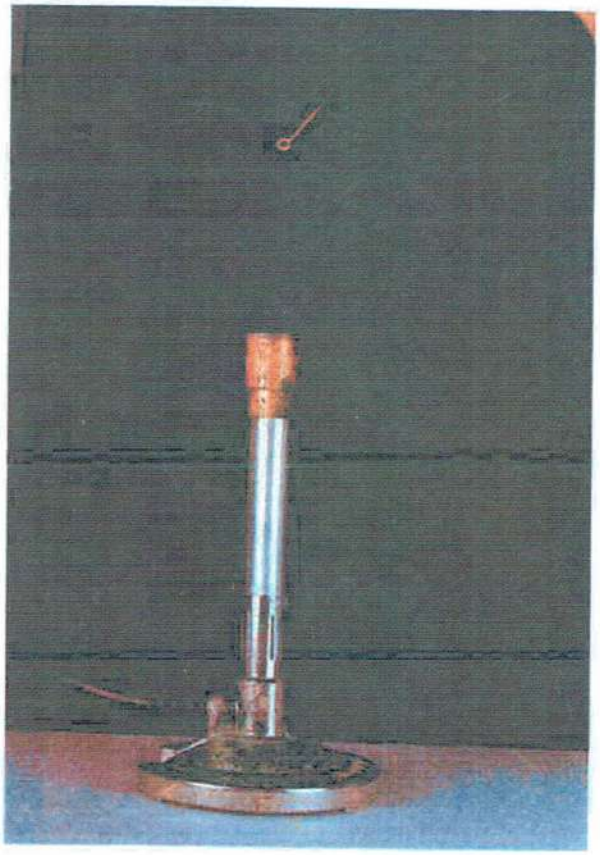

FIGURE 4.53 Sterilizing a loop in a Bunsen burner flame. The end of the wire and the entire loop must be held in the flame until they glow red.

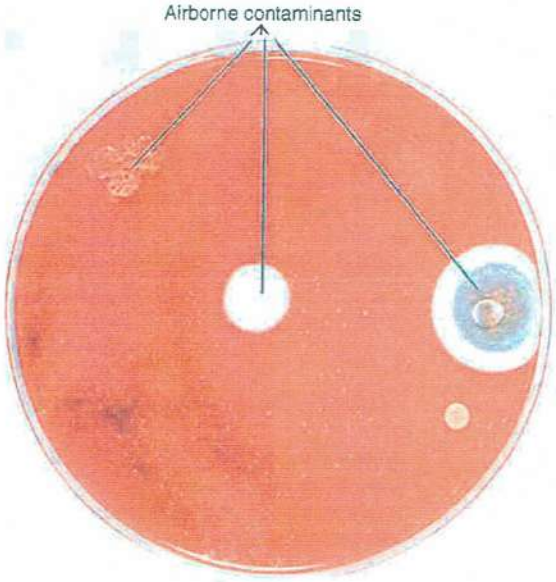

FIGURE 4.54 Contamination of a streak plate, resulting from leaving the plate open too long.

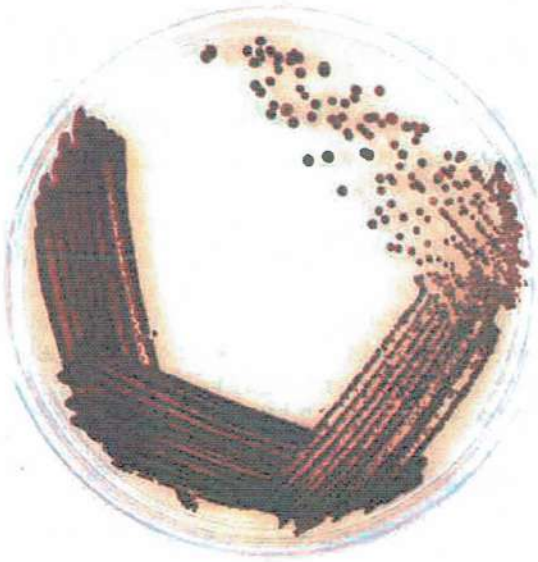

FIGURE 4.55 A streak plate of *Chromobacterium violaceum* on nutrient agar, illustrating expected results.

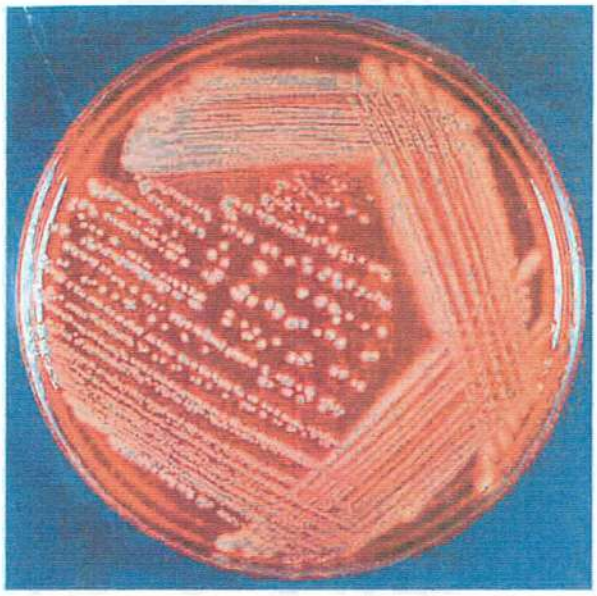

FIGURE 4.56 A streak plate of *Alcaligenes faecalis* on blood agar. Colonies are circular, entire, umbonate, smooth, shiny, moderate, white, and translucent.

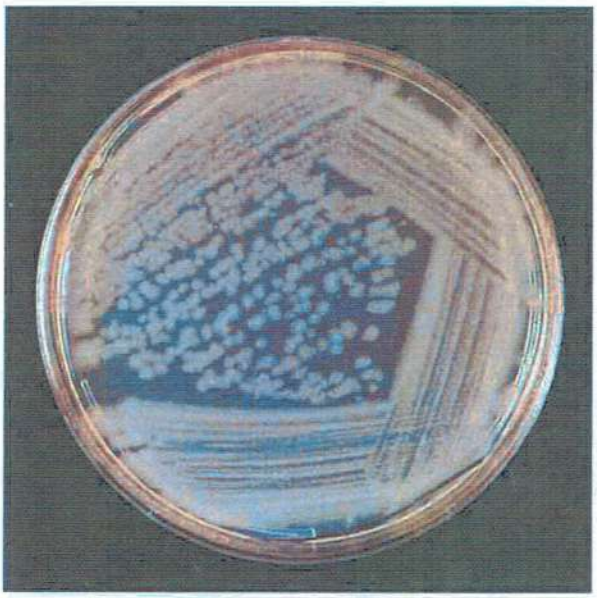

FIGURE 4.57 A streak plate of *Bacillus cereus* on nutrient agar. This organism produces colonies that are irregular, entire, raised, rough, dull, large, tan, and opaque.

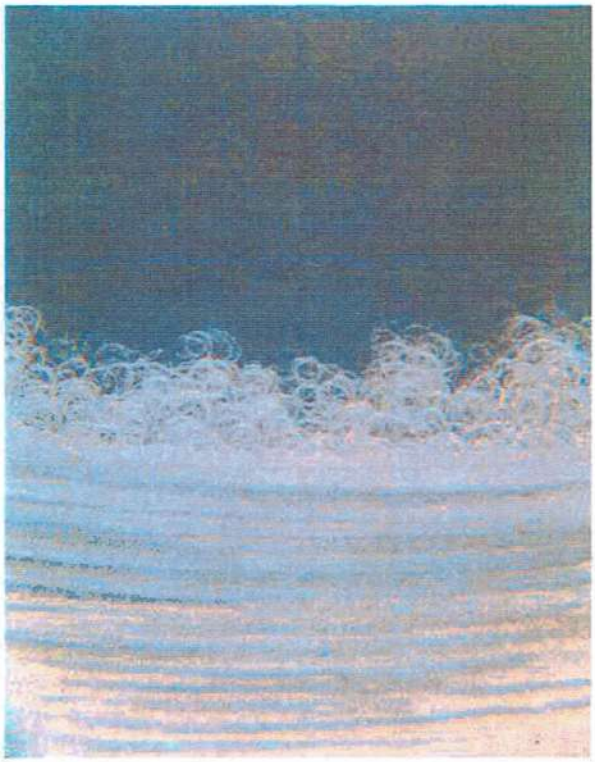

FIGURE 4.58 The edge of a *Bacillus circulans* colony on nutrient agar. This organism produces colonies similar to *Bacillus cereus* (see Figure 4.57), but with filamentous margins that curl (3×).

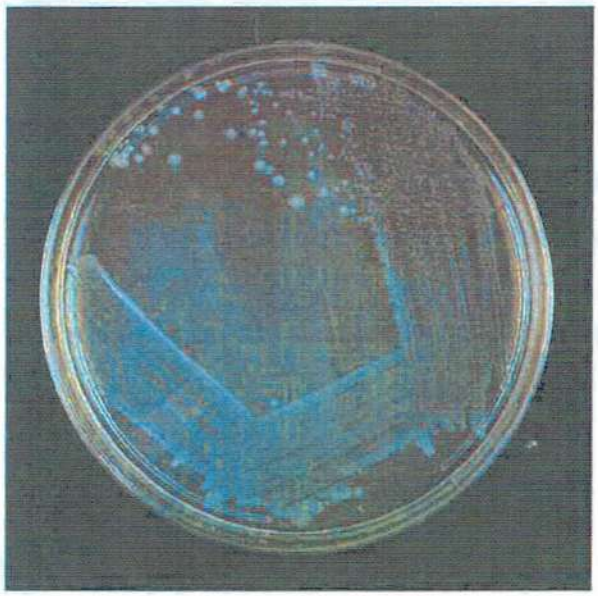

FIGURE 4.59 A streak plate of *Citrobacter freundii* on nutrient agar. Colonies are circular, entire, convex, smooth, shiny, moderate, cream-colored, and translucent.

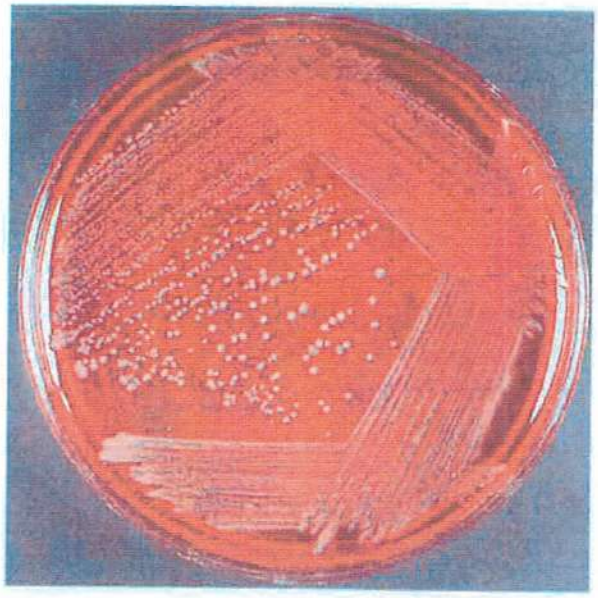

FIGURE 4.60 A streak plate of *Enterobacter aerogenes* on blood agar. Colonies are circular, entire, convex, smooth, shiny, moderate, white, and opaque.

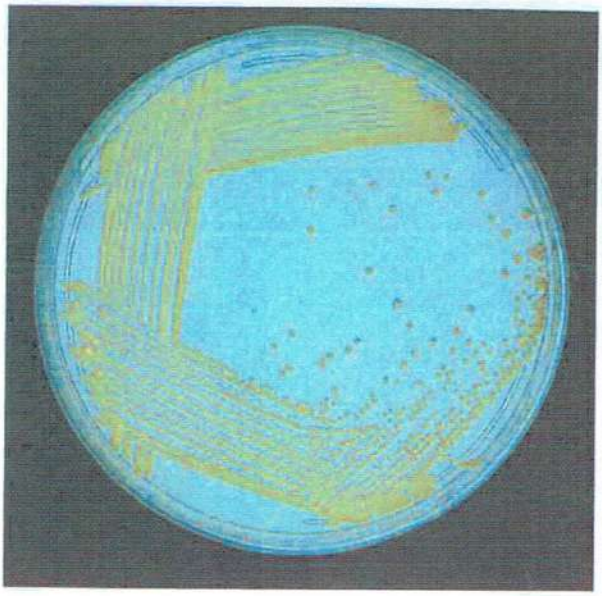

FIGURE 4.62 A streak plate of *Micrococcus luteus* on nutrient agar. Colonies are circular, entire, convex, smooth, shiny, small, yellowish, and opaque.

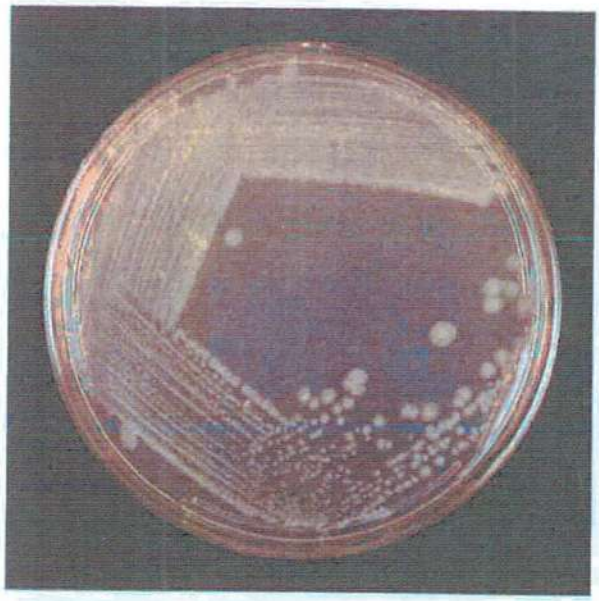

FIGURE 4.61 A streak plate of *Escherichia coli* on nutrient agar. *E. coli* colonies are circular, entire, convex, smooth, shiny, large, tan, and translucent.

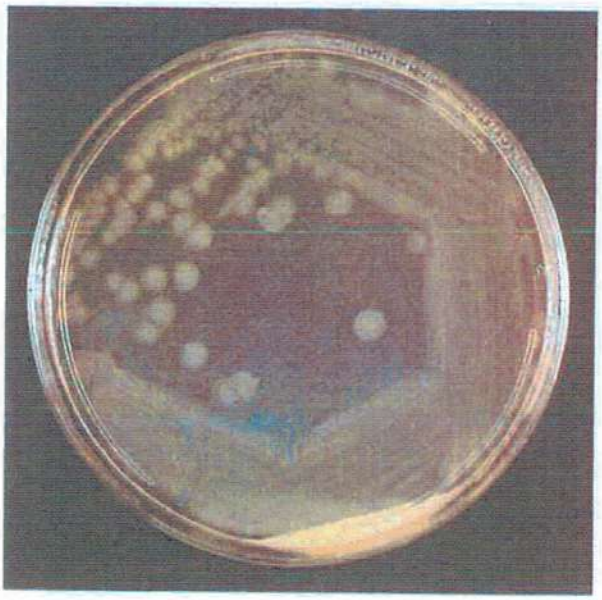

FIGURE 4.63 A streak plate of *Pseudomonas aeruginosa* on nutrient agar. On this medium, colonies are circular, entire, raised, rough, shiny, large, tan, and translucent.

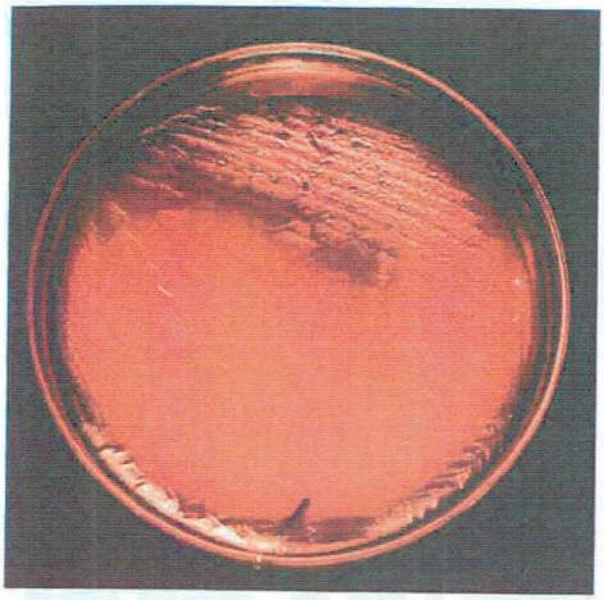

FIGURE 4.64 A streak plate of *Pseudomonas aeruginosa* on blood agar. On this medium, *P. aeruginosa* forms large, flat colonies with a glistening appearance (compare to Figure 4.63).

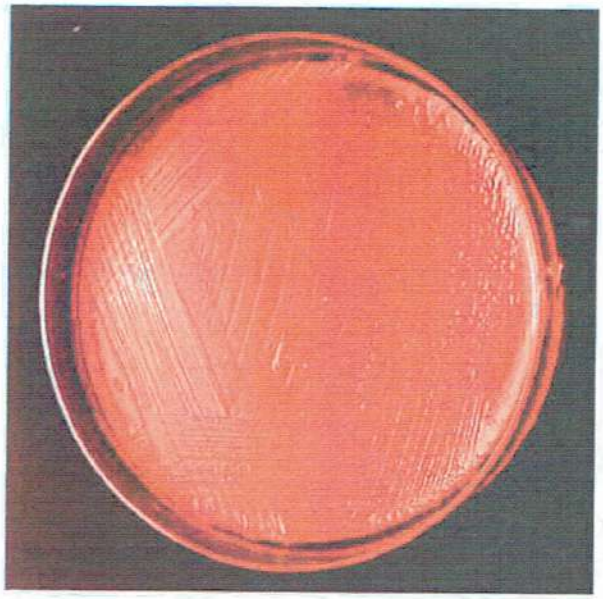

FIGURE 4.66 A streak plate of *Proteus vulgaris* on blood agar. This organism forms colonies with outward-extending waves, a result of periodic migration of cells across the agar surface. This growth pattern on agar, called swarming, is characteristic of *Proteus*.

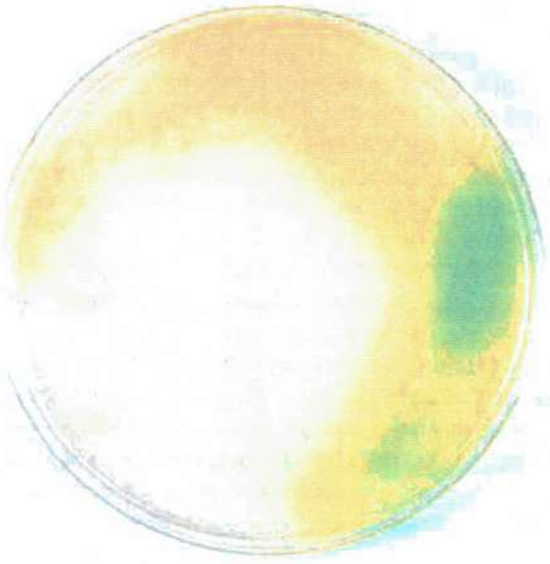

FIGURE 4.65 A streak plate of *Pseudomonas aeruginosa* on Mueller Hinton agar. On this medium, *P. aeruginosa* produces a readily apparent, green, diffusable pigment (compare to Figures 4.63 and 4.64).

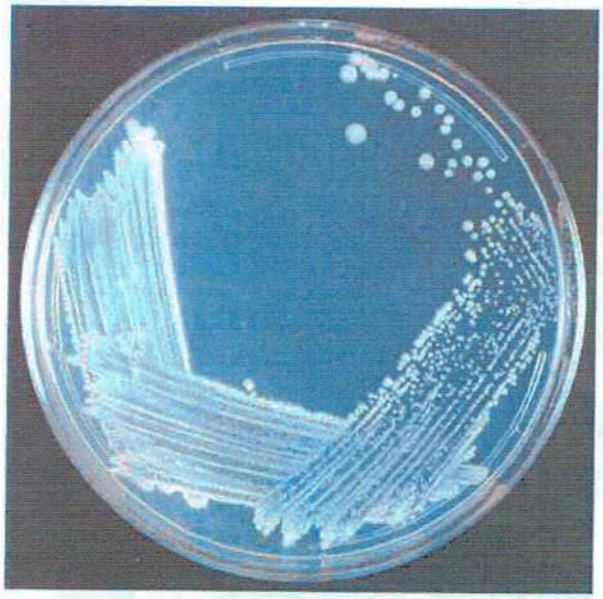

FIGURE 4.67 A streak plate of *Staphylococcus aureus* on nutrient agar. Colonies are circular, entire, convex, smooth, shiny, moderate, cream-colored with a yellowish tint, and opaque.

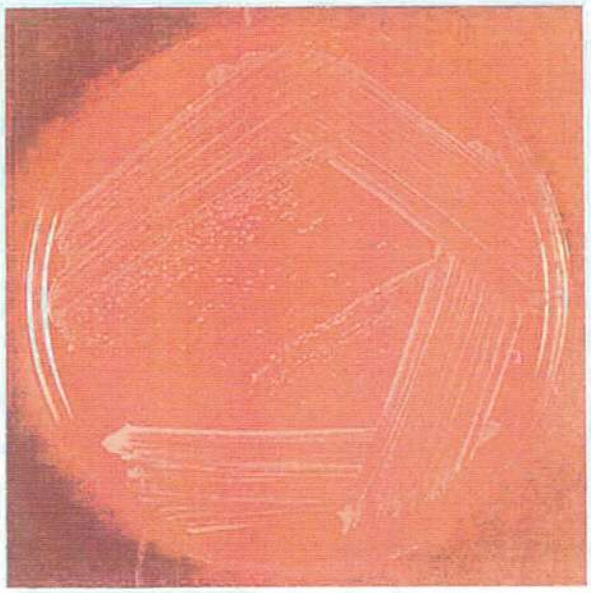

FIGURE 4.68 A streak plate of *Staphylococcus epidermidis* on blood agar. This organism produces colonies that are circular, entire, convex, smooth, shiny, pinpoint, white, and opaque.

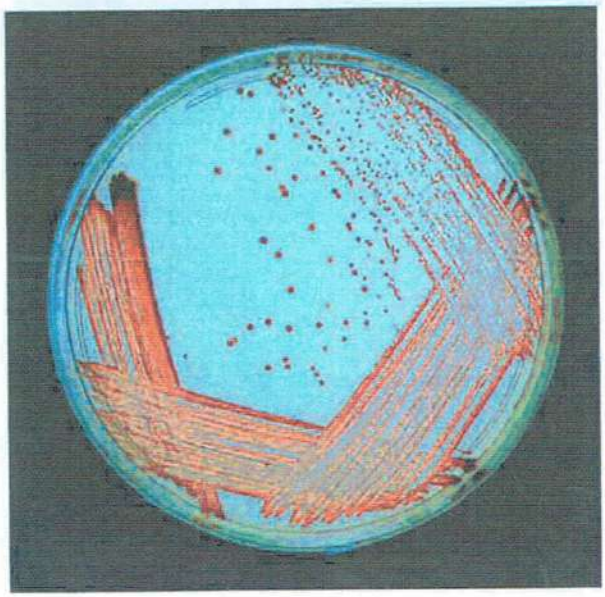

FIGURE 4.69 A streak plate of *Serratia marcescens* on nutrient agar. Colonies are circular, entire, convex, smooth, shiny, small, red, and opaque.

MAINTENANCE OF BACTERIAL CULTURES

FIGURE 4.70 Blood agar slants for short-term storage of bacterial cultures.

During growth on agar or in broth, bacteria use up available nutrients and produce toxic products that accumulate in the medium. Therefore, bacteria must be transferred periodically to fresh media in order to maintain their viability. For most cultures, transfer should occur every 3–4 weeks.

Transferring bacteria to a general purpose medium, such as nutrient agar, is often effective, but transferring to blood agar is even better, because blood contains nutrients used by most bacteria. Also, transferring to **slants** (Figure 4.70) is more effective than transferring to plates, because media are less likely to dry out when in tubes. After 24 to 48 hours of incubation immediately following transfer, storing slants in the refrigerator will prolong the life of the culture.

References:

(1) Michael J. Leboffe. Burton E. Pierce. (2016) A photographic Atlas for the Microbiology Laboratory. 5th Edition.

(2) FAIRBROTHER, R.W. (2013). **A Textbook of Bacteriology, Fourth Edition.**