Bio 139 Microbiology Lab #4, #5
Media & Aseptic Technique.

You must wear a lab coat.

1. Growth of bacteria on artificial medium
   a. in contrast to natural medium (pond water, tissue)
   b. grow in lab to study genetics and biochemistry of bacteria

Heterotrophs: require organic sources of carbon/energy (e.g., glucose)
Autotrophs: use CO₂ as sole carbon source (soil, water microbes)

2. Medium: nutrient mixture that serves as a growth substrate
   a. all media must provide the proper nutrients as well as physical and chemical environment for growth of a specific bacterial species
      1. different species have very different requirements

3. Composition of media
   a. defined medium: the exact composition and mM concentration of each component is known.
   b. undefined medium: composition and concentration of some of the components are not known
      e.g., cell extracts: the exact concentration of each component is not known (complex mixture).
      Any recipe containing “yeast extract”, peptone, etc. is undefined.

4. Media recipes: component amounts often expressed as % (g/100 mL), or g/L
   How many grams yeast extract is needed to prepare 500 mL of medium containing 0.5% yeast extract? ➔ 2.5 g % times (#100 mL)

5. Physical state of media
   a. Liquid media = broth
   b. Solid media = agar (plates)
      1. Agar is solidifying agent (derivative of marine algae). It is a complex carbohydrate NOT metabolized by most bacterial species. It does not serve as a nutrient source (stays solid!).

6. Media must be sterilized
   a. Sterilization: killing/removal of all microorganisms from a given material or object (including endospores)
      1. Necessary due to the ubiquitous presence of microbes in all environments
   b. Autoclave: moist heat with pressure: 15 minutes, 121°C, 15 lb/in²
      1. Autoclaving also serves to melt agar
         24°C agar = solid (powder)
         100°C agar = liquid
         42°C agar resolidifies
         Add agar powder to broth, autoclave, cools to form a solid medium
   c. Pasteurization: heating to 62.9°C for 30 minutes; kills disease causing bacteria, but not all bacteria.
      Therefore milk is not sterile; it will spoil due to the presence of non-pathogenic bacteria
7. Applications of media
   a. **Broth**: grow large numbers of cells ($10^9$-$10^{12}$)
   b. **Agar**: grow individual colonies to obtain **pure culture**
      This makes identification of bacterial species possible. CRUCIAL in this course!

8. Agar (solid media) is used to produce **colonies**
   a. Bacterial cells can be separated on agar surface
   b. Individual cells attach to the surface and begin to divide (reproduce)
   c. The progeny of a single cell will form a visible mass fixed to the agar surface
      1. This colony contains about 200,000-500,000 descendents of the original cell
      2. Therefore, all cells in a colony are genetically and biochemically identical (clones of the original cell)

Colonies are used to identify the species.
   a. Each species produces unique colony morphologies and biochemical reactions
   b. Mixed cultures cannot be us