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Microbiology Lab

Ubiquity of Microorganisms: Microorganisms are ubiquitous; that is, they are present nearly everywhere. In this lab you will try to isolate bacteria and other microorganisms from various sources using different types of media.

Terms and definitions

**Culture media** (medium, singular): Solution of nutrients required for growth of bacteria.

**Agar:** a carbohydrate derived from seaweed used to solidify a liquid medium

**Tryptic Soy Agar (TSA):** a rich solid medium containing a digest of casein (the principal milk protein) and soy products. It is an all-purpose medium that supports the growth of many diverse organisms.

**Colony:** a visible population of microorganisms originating from a single parent cell and growing on a solid medium.

**PROCEDURE:** (Work in Student pairs)

1. Collect a small TSA plate and write your name and class period on the back of the plate with a permanent marker. Also, write down the object you will be swabbing for bacteria on the back of your plate (e.g. doorknob)

2. Moisten a sterile swab with sterile water. Using this swab, collect a sample from any surface or object (e.g. doorknobs, shoes, drinking fountain, strand of hair, various body parts, etc.) Try whatever interests you and be creative!

3. After the sample has been collected, inoculate the TSA plate by gently rolling the swab over the surface of the agar. Each student should have their own swab and inoculate their own plate with their swab.

4. Replace the lid on the plate and place in the purple basket in the front of the room. Plates are always incubated in an inverted position (Agar side up)

5. Incubation: Wait two days. Bacteria will grow at room temperature.

**STAINING:**

Background:

The most important staining technique in Bacteriology is the Gram stain. The Gram stain is a differential stain commonly used in the laboratory that differentiates bacteria on the basis of their cell wall structure. Most bacteria can be divided into two groups based on the composition of their cell wall:

1. Gram-positive cell walls have a thick layer of peptidoglycan beyond the plasma membrane. Gram-positive cell walls stain blue/purple with the gram stain
2. Gram negative cell walls have a thin peptidoglycan layer and an outer membrane beyond the plasma membrane. Gram negative cells will stain pink with the Gram stain.

Procedure: (EACH STUDENT)

1. Using an eyedropper, add 1 drop of sterile water to the slide.
2. Next, sterilize the inoculating needle and add a colony of the bacteria growing on your plate.
3. Air dry and Heat Fix
4. Cover the smear with crystal violet (primary stain) for 1 minute.
5. Gently wash off the slide with water.
6. Add iodine (mordant) for 1 minute.
7. Wash with water.
8. Decolorize with alcohol for 10-15 seconds. This is the tricky step. Stop decoloring with alcohol as soon as the blue color has stopped leaching off the slide and then immediately was with water.
9. Add the counterstain, safranin for 30 seconds
10. Wash both the top and the bottom of the slide with water.
11. Blot the slide with bibulous paper or allow to air dry.

USING A MICROSCOPE to observe the bacteria

Hypothesis (3 pts):

1. What is TSA and what does it allow to happen (2 pts)?

2. Draw the peptidoglycan layers in a Gram positive and a Gram negative cell (4 pts).

3. What type of bacteria did you have on your plate (Gram + or Gram -)? Draw what you saw under the microscope. (2 pts)

4. Analyze and describe the difference between cocci and bacilli bacteria (4 pts)