LAB 2
MICROORGANISMS IN THE LABORATORY: COLLECTION AND ISOLATION OF "UNKNOWN" BACTERIA

Objectives

- In this lab you will learn how to:
  - collect microorganisms from the skin or from your cellphone
  - use aseptic techniques to handle and transfer bacteria
  - conduct a simple stain of bacteria
  - isolate a pure culture of bacteria for subsequent identification
  - describe the appearance of bacterial colonies growing on artificial media

Introduction

Microbes are everywhere, and can be easily collected from air, water, food, soil, and your own body. Microbiologists routinely face the challenge of collecting and isolating pure cultures of these microbes for study. Once collected, the microbes can be grown on a **culture medium**, a liquid or solid that contains the nutrients necessary for microbial survival and growth. The culture medium must first be sterilized, then inoculated with the microbe and incubated at the appropriate temperature for a certain amount of time (usually 24-48 hours). Microbes collected from your body will grow best at human body temperature, or 37°C (98.6°F).

Solid culture media require the addition of a solidifying agent, such as **agar**. Agar is a polysaccharide derived from marine algae. Most bacteria cannot digest it, which is a good thing because otherwise, the medium would be liquified as the bacteria digested it! Most solid culture media, such as **nutrient agar**, contain 1.5% agar in addition to various nutrients. Because agar liquifies at 100°C and resolidifies at 40°C, an **agar deep** (a tube of previously-prepared culture medium) can be melted and poured into an empty, sterile **Petri dish**. A Petri dish holds 15-20mL of medium and provides a large surface area for bacterial growth and isolation. Once in the Petri dish, the medium will solidify as it cools and remain solid at common incubation temperatures.

On a **Petri plate** (a Petri dish filled with culture medium), bacteria will grow and multiply to form colonies. A bacterial colony often looks like a small dot or streak on the medium, and contains *millions* of individual cells. A single species of bacteria growing in culture medium is considered a **pure culture**.

Part I: Pouring Petri Plates and Inoculating the Culture Medium

Each person will make a Petri plate to collect a bacterial sample from their skin. Make sure you know which side of the empty Petri dish is "up," before pouring the melted medium into it!

Procedures

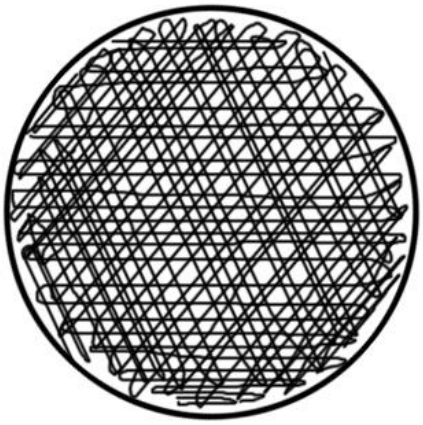
Obtain:

- melted nutrient agar deep (from the beaker on the hot plate)
- sterile Petri dish
- sterile disposable swab

Handle the melted nutrient agar deep carefully, because it can burn you! Use a test tube clamp to handle the hot tube. You might want to put the hot tube into an empty aluminum can, then take it back to your desk, along with a Petri dish and a disposable swab.

Label the BOTTOM of the Petri dish with your initials. Flip it right-side up, open the top and pour the melted agar into it. Let it cool (this should take 15 minutes, or until the medium looks slightly translucent). To keep unwanted microbes out, always **keep the lid on**, unless you are inoculating the Petri plate or sampling from it.

Once it has cooled, you are ready to sample some bacteria from your skin. Remove the sterile swab from its wrapper, streak it across your skin somewhere on your body. Next, streak the swab on the surface of the agar, back and forth in a zig-zag pattern, as shown below:



Incubate this plate upside down for 48 hours at 37°C.

Why do you suppose the Petri plate should be inverted when it is in the incubator?

Why is it being incubated at 37°C?

Part II: Aseptic Transfer of Bacteria and Simple Staining

Next you will make a slide of a common laboratory bacterium (*E. coli* or *Bacillus megaterium*), stain it, and examine it under the microscope.

Procedures

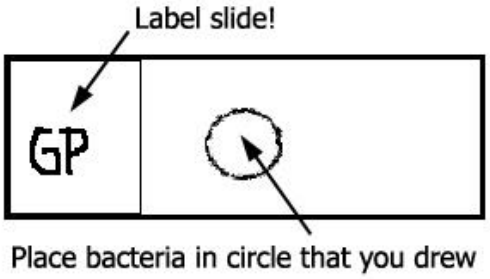
Obtain:

- tube of bacteria (EC = *E. coli* ; or BM = *Bacillus megaterium*)
- new, cleaned slide
- transfer loop
- burner
- striker to light the burner
- clothespin
- methylene blue or crystal violet stain

Clean your slide well, then rinse and dry it thoroughly. Mark a small circle on the slide with a marker – this is where you will put the bacteria. Actually, the side that is marked will be the bottom of the slide, so put the bacteria on the other side (top) of the slide. Don't forget to label each slide according to the bacterial culture used.

Next, gently swirl the tube of bacteria to suspend the cells. **Flame sterilize** the end of the transfer loop as demonstrated by your instructor. Allow the transfer loop to cool down (about 30 seconds), without contaminating or touching the loop to any surface, such as the desktop. Open the cap of the tube with the bacteria in it, dip the loop in, and transfer a small droplet of bacteria to the slide. Your instructor will demonstrate the right way to do this.

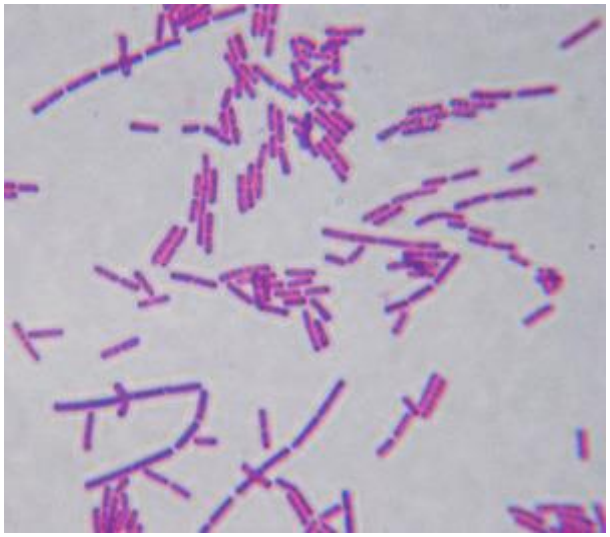
Immediately flame sterilize the transfer loop again, without contaminating or touching the loop to any surface, such as the desktop! Only then can you set the transfer loop down on your desk.



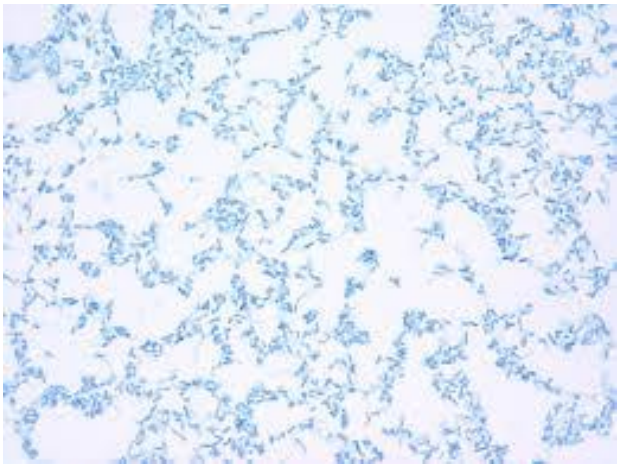
Allow the small droplet containing bacteria to **dry** on the slide with the slide warmer.

Using a clothespin to handle the slide, **heat fix** the bacteria to the slide by waving the slide through the flame 3 times (1 or 2 seconds each pass). Next, apply enough stain to cover the circle you drew on the slide. Let it sit for 30 seconds, then wash the stain off under the faucet, using a very gentle stream of water. Again, dry the slide. You can gently blot away the excess water outside of the circle, but do not rub or touch the bacteria inside the circle.

Once the slide is completely dry, examine it under low power (there is no coverslip!), then work your way up to 1000X magnification with oil immersion.



Bacillus megaterium **bacteria stained with crystal violet (1000X).**



E. coli **bacteria stained with methylene blue (1000X).**

Part III: Isolating Bacteria and Describing Colony / Cell Morphology

Next week you will examine your Petri plates in more detail, and stain and look at the bacteria that you collected from your skin.