University of Samarra
College of science applied
Department of biotechnology

Biotechnology
Principle of Biotechnology/ lab

Lab no.: 3

The Isolation of Microorganisms from a different environments by a different techniques

Objective: A students should be able to understand the different Isolation techniques & the purpose of the Pure Cultures Isolation.

The survival, growth & the ecological distribution of microorganisms is greatly affected by the chemical and physical nature of their environment. Therefore M.O. can be isolated from many different environments such as soil, water, air, food, plants & animals. (**M.O. grow and divide as rapidly as the environment permits)**
When working with microorganisms, it is desirable to start with single, isolated colonies to ensure you are working with a pure culture, also In order to adequately study and characterize an individual M.O. species, one needs a **pure culture.**
The colony theoretically forms from a single cell, a colony should then represent a pure culture. A **colony** is a visible mass of microorganisms growing on an agar surface and usually originating from a single organism or arrangement of organisms.

Three different techniques can be used to obtain pure colonies or pure isolates:

1- The **spread plate technique** is an easy, direct way of achieving this result. In this technique, a small volume of dilute bacterial mixture containing 100 to 200 cells or less is transferred to the center of an agar plate and is spread evenly over the surface with a sterile, L-shaped glass rod. After incubation, some of the dispersed cells develop into isolated colonies. In this procedure, one assumes that a colony is derived from one cell and therefore represents a clone of a pure culture. After incubation, the general form of the colony and the shape of the edge or margin can be determined by looking down at the top of the colony. The nature of the colony elevation is apparent when viewed from the side as the plate is held at eye level. These variations are illustrated in figure1. After a well-isolated colony has been identified, it can then be picked up and streaked onto a fresh medium to obtain a pure culture.

2- The **streak-plate technique** in this technique, the bacterial mixture is transferred to the edge of an agar plate with an inoculating loop and then streaked out over the surface in one of several patterns. At some point on the streaks, individual cells will be removed from the loop as it glides along the agar surface and will give rise to separate colonies figure 2. Again, one assumes that one colony comes from one cell. The key principle of this method is that by streaking, a dilution gradient is established on the surface of the plate as cells are deposited on the agar surface. Because of this gradient, confluent growth occurs on part of the plate where the cells are not sufficiently separated, and individual, well isolated colonies develop in other regions of the plate where few enough cells are deposited to form separate colonies that can be seen with the naked eye.

Cells from the new colony can then be picked up with an inoculating loop or needle and transferred to an agar slant or other suitable medium for maintenance of the pure culture.

3- The **pour-plate technique** also will yield isolated colonies and has been extensively used with bacteria and fungi. The original sample is diluted several times to reduce the microbial population sufficiently to obtain separate colonies upon plating figure 3.
The small volumes of several diluted samples are added to sterile petri plates and mixed with liquid tryptic soy agar that has been cooled to about 48° to 50°C. Most bacteria and fungi will not be killed by the brief exposure to the warm agar. After the agar has hardened, each cell is fixed in place and will form an individual colony if the sample is dilute enough. Assuming no chaining or cell clusters, the total number of colonies are equivalent to the number of viable microorganisms in the diluted sample. To prepare **pure cultures,** colonies growing on the surface or subsurface can be inoculated into fresh medium.

Materials & Procedure
Brain heart infusion agar
95% ethyl alcohol
L-shaped glass rod
Petri plates
Inoculating loop
Bunsen burner
Sterile pipettes
Soil sample
Water sample

The spreading Procedure
1. Pipette 0.1 ml of the water sample or serial dilutes of soil sample onto the center of BHI agar plate.
2. Dip the L-shaped glass rod into a beaker of ethanol and then briefly pass the ethanol-soaked spreader through the flame to burn off the alcohol and allow it to cool inside the lid of a sterile petri plate.
3. Spread the sample evenly over the agar surface with the sterilized spreader, making sure the entire surface of the plate has been covered.
4. Invert the plates and incubate for 24 to 48 hours at room temperature or 30°C.
5. After incubation, measure some representative colonies and carefully observe their morphology. Record your results in the report for lab 3.

The streaking Procedure

1. Aseptically remove a loopful of the water or serial dilute of soil sample.
2. Streak out the loopful of sample on the BHI agar plate that you have prepared as follows:
a. Carefully lift the top of the petri plate just enough to insert your inoculating loop easily, in order to avoid contamination. Insert the inoculating loopful of sample and spread it
Over a small area (area 1) at one edge of the plate as shown in figure 2.
b. Remove the inoculating loop and kill any remaining bacteria by flaming them. Then
Insert the loop under the lid and cool it at the edge of the agar near area 1.
c. Rotate the plate while carefully keeping in mind where the initial streaks ended (use
The marked quadrants as a guide) and cross over the streaks in area 1.
d. Remove the loop, flame it, cool in the agar as before, and repeat the streaking process.
3. Incubate the plates at 30° to 37°C for 24 to 48 hours in an inverted position. Afterwards, examine each of the agar plates to determine the distribution and amount of growth in the three or four streaked areas and record your results in the report for lab 3.

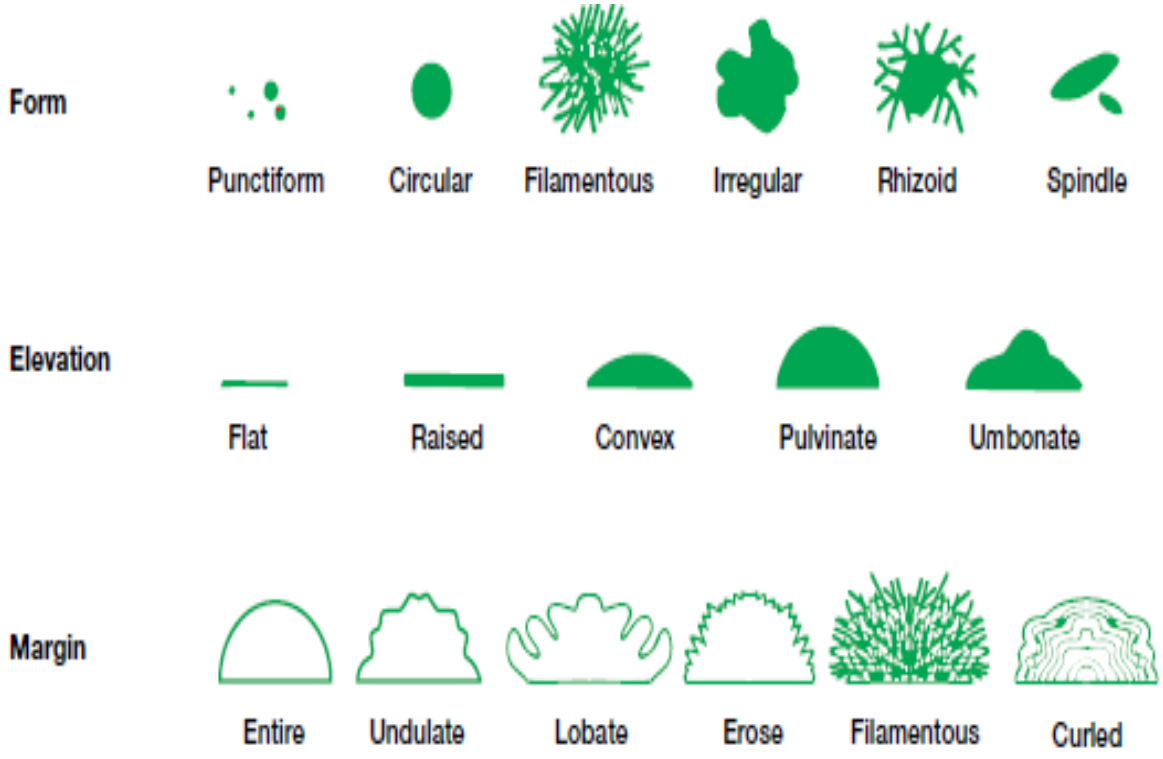

Figure 1: **Bacterial Colony Characteristics on Agar Media as Seen with the Naked Eye.** The characteristics of bacterial colonies are described using the following terms.
Appearance: Shiny or dull Optical property: Opaque, translucent, transparent
Pigmentation: Pigmented (purple, red, yellow) Non-pigmented (cream, tan, white)
Texture: Rough or smooth

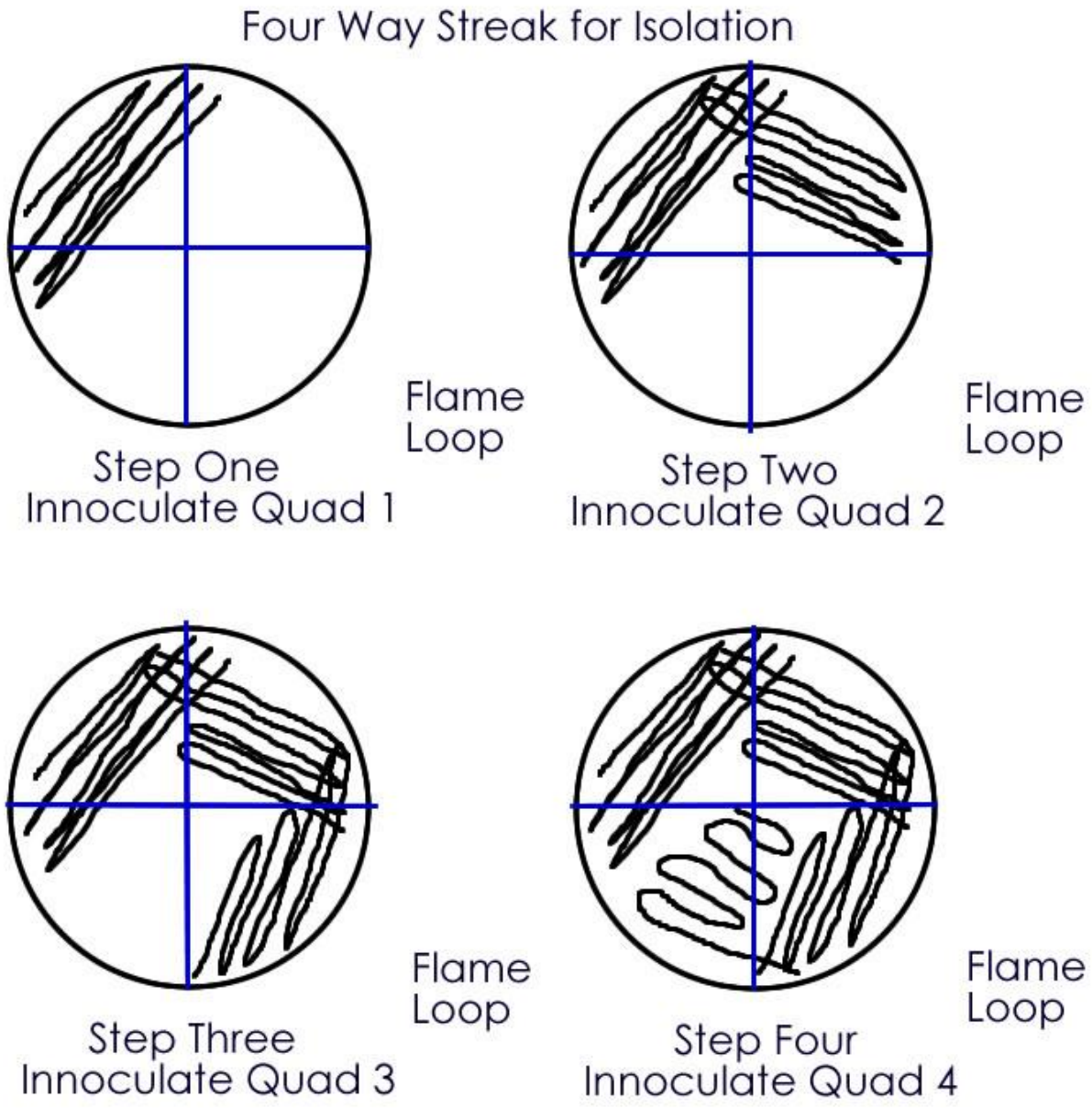

Figure 2: The **streak-plate technique**

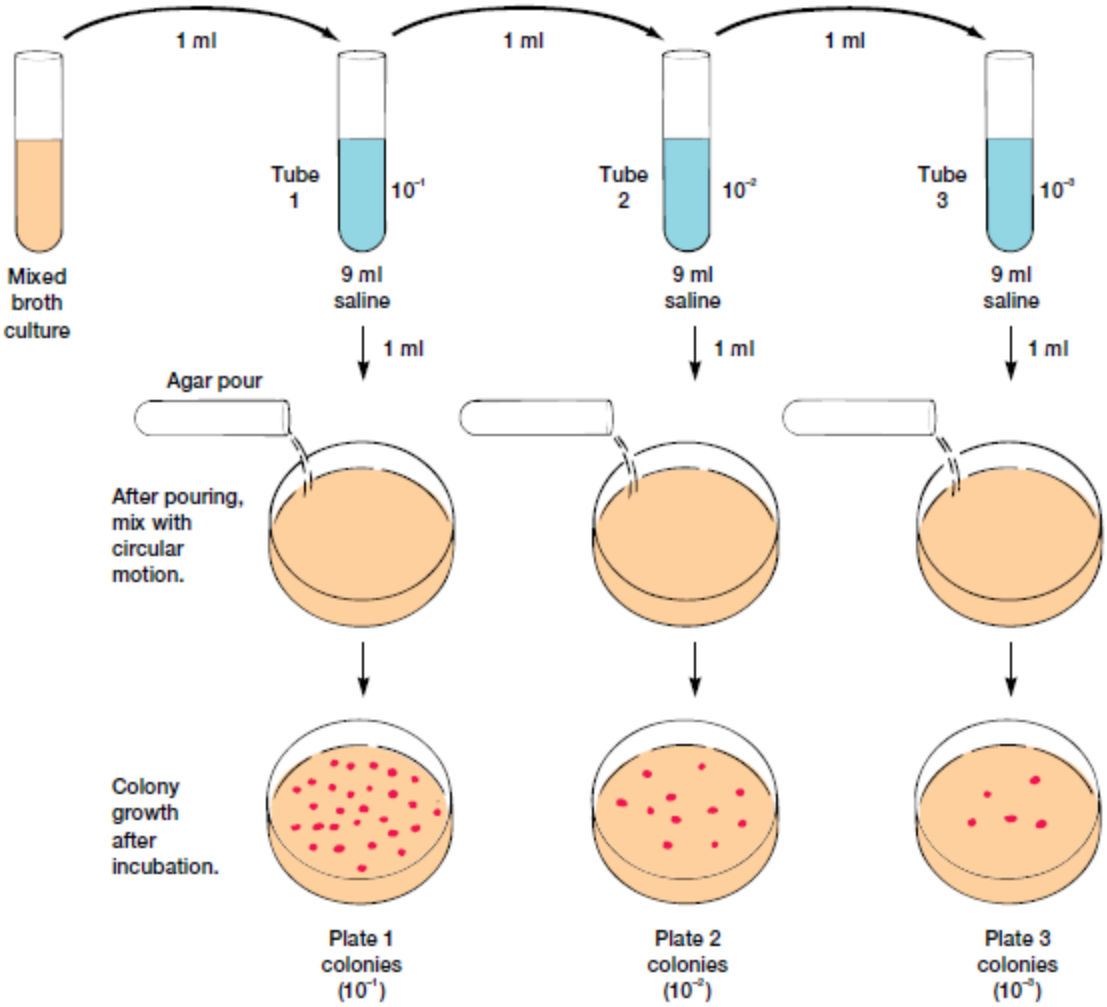

Figure 3: The Pour-Plate Technique. The original sample is diluted several times to decrease or dilute the population sufficiently. 1 ml of each dilution is then dispensed into the bottom of a petri plate. Agar pours are then added to each plate. Isolated cells grow into colonies and can be used to establish pure cultures. The surface colonies are circular and large, subsurface colonies are lenticular or lens shaped and much smaller.