EXPERIMENT 1

BASIC MICROBIOLOGICAL TECHNIQUES FOR ISOLATION, CULTIVATION, AND CULTURAL CHARACTERIZATION

Figure 1: Streaked agar plates (Source: https://en.wikipedia.org/wiki/Streaking_(microbiology)/)

TOPIC OUTCOMES:

Student should be able to

1. To familiarize with the equipment and materials needed to develop isolation or culturing of microorganism.
2. To understand and demonstrate isolation and culturing using streak plate and spread plate for separation of mixed microorganisms to individual colonies.
3. To understand and differentiate morphological and characteristic of microorganism grown in pure culture.

MATERIAL

- Microorganism (s) - two types of microorganism are given and already streaked on an agar plate. (Each group will be given two plates)
- Bunsen burner (1 for each group)
- Nutrient broth (4 for each group)

- Nutrient slant agar (4 for each group)
- Nutrient agar deep tube (4 for each group)
- Nutrient agar plates (4 for each group)
- Wire loop (1 for each group)
- Needle end loop (1 for each group)
- L-shaped Bend rod (1 for each group)
- 50-mL 70% Ethanol (1 Bottle each group)

METHODOLOGY

1) Culture transfer technique

   a) You are provided with two types of microorganisms, two nutrient broths, two nutrient agar slants, and two nutrient agar deep tubes.
   b) Label the tubes provided accordingly based on the types of microorganism used.
   c) Flame the wire loop until the entire loop or tip wire is red.
   d) Let it cool and then touch a single colony from the agar plates (containing microbes) and transfer it into the nutrient broth. Make sure mix a little bit to ensure the microbes mix with the medium.
   e) Repeat step (c), but this time transfer it to the slant agar tube on the surface of the agar and make a zig zag motion with the loop.
   f) Next, repeat step (c), but instead of using wire loop used a needle-end wire to pick up a colony from agar plate and stabbed it into the agar deep tube but make sure that it is done until almost ¾ of the agar.
   g) Gather all tubes and incubate them in 37 C incubator for 24 hrs.

2) Isolation of pure cultures

   2.1 Streak plate

   a) You are provided 24-hour culture broth containing mixed cultures.
   b) By using the same culture transfer technique shown above, wire loop is flame and cool, but this time the loop is used to take liquid sample by lightly immerse the tip of the loop to get a loop of liquid.
   c) Transfer to an agar plate by dragging it several times across the area A (shown in Figure 2).
   d) Then, the wire loop is again flamed until it becomes red and cool down before touching the loop on area A and dragged it several times at area B.
   e) The loop is flamed again and cools down before touching the loop on area B and dragging it several times at area C.
   f) The steps are repeated to create the final area 4.
   g) Gather the agar plates and incubate them in 37 C for 24 hours. Do in duplicates.

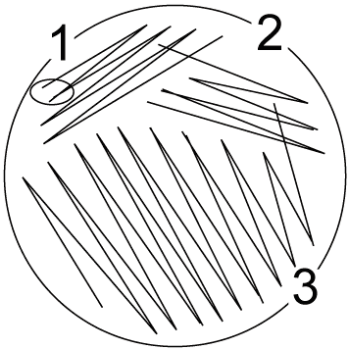

Figure 2: Streaking steps for agar plates (Source: https://www.gene.affrc.go.jp/manual.html/)

2.2 Spread plate (Figure 3)

a) From the mix culture provided take 10 ml and add on the middle of an agar plate.
b) In the meantime, place a sterile L-shaped bent rod in 70 % Ethanol to cover the bend part of the rod.
c) Take out the rod and pass through the flame to burn off the alcohol and cold it on the agar plate as shown by the demonstrator.
d) After 15 seconds, remove the lid of the agar plates, and with one hand turning the plate, the other hand is holding the bend rod on the surface to spread the liquid culture.
e) Make sure that the liquid is totally spread before incubating the agar plates in 37 C for 24 hours.

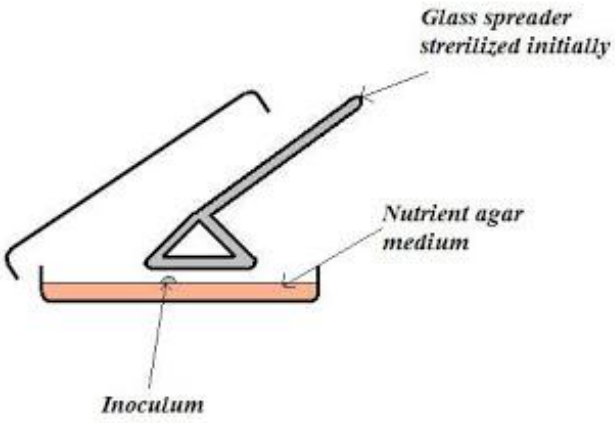

Figure 3: Spread plate method (Source: http://principlemicrobiology.blogspot.my/2008/04/spread-plate-method)

2.3 Characteristic of microorganism

Observe the tubes and agar plates and determine the characteristic of the microorganisms either known or unknown microbes available. Compare the observation with the examples in Figure 4.

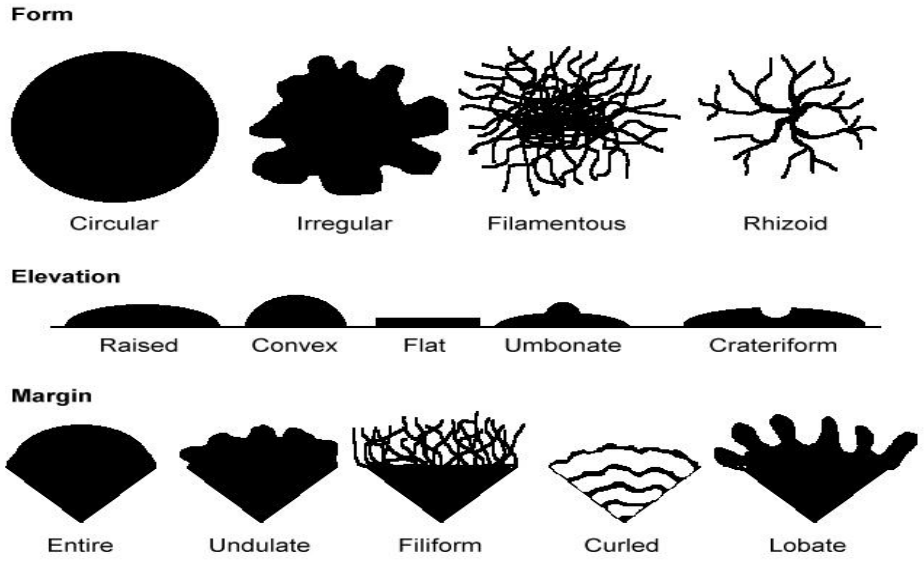

Figure 4: Characteristic of microorganisms on agar plates. (Source: http://microbeonline.com/)

QUESTIONS

1. Explain the difference between enriched, selective and differential media for growing microorganisms.
2. Write down selective medium used to cultivate one microorganism of your choice.