Lab 3: Obtaining Pure Cultures from a Mixed Population

DISCUSSION

As stated in Lab 2, microorganisms exist in nature as mixed populations. However, to study microorganisms in the laboratory we must have them in the form of a pure culture, that is, one in which all organisms are descendants of the same organism. Two major steps are involved in obtaining pure cultures from a mixed population:

1. First, the mixture must be diluted until the various individual microorganisms become separated far enough apart on an agar surface that after incubation they form visible colonies isolated from the colonies of other microorganisms. This plate is called an isolation plate.

2. Then, an isolated colony can be aseptically "picked off" the isolation plate (Figure 1) and transferred to new sterile medium (see Fig. 3). After incubation, all organisms in the new culture will be descendants of the same organism, that is, a pure culture.
A. STREAK PLATE METHOD OF ISOLATION

The most common way of separating bacterial cells on the agar surface to obtain isolated colonies is the streak plate method we used in Lab 2 to inoculate a petri plate. It provides a simple and rapid method of diluting the sample by mechanical means. As the loop is streaked across the agar surface, more and more bacteria are rubbed off until individual separated organisms are deposited on the agar. After incubation, the area at the beginning of the streak pattern will show confluent growth while the area near the end of the pattern should show discrete colonies (see Fig. 2A and Fig. 2B).

B. THE POUR PLATE AND SPIN PLATE METHODS OF ISOLATION

Another method of separating bacteria is the pour plate method. With the pour plate method, the bacteria are mixed with melted agar until evenly distributed and separated throughout the liquid. The melted agar is then poured into an empty plate and allowed to solidify. After incubation, discrete bacterial colonies can then be found growing both on the agar and in the agar.

The spin plate method involves diluting the bacterial sample in tubes of sterile water, saline, or broth. Small samples of the diluted bacteria are then pipetted onto the surface of agar plates. A sterile, bent-glass rod is then used to spread the bacteria evenly over the entire agar surface (see Fig. 4) in order to see isolated colonies (see Fig. 5). In Lab 4 we will use this technique as part of the plate count method of enumerating bacteria.

C. USE OF SPECIALIZED MEDIA

To supplement mechanical techniques of isolation such as the streak plate method, many special-purpose media are available to the microbiologist to aid in the isolation and identification of specific microorganisms. These special purpose media fall into four groups: selective media, differential media, enrichment media, and combination selective and differential media.

1. Selective media: A selective medium has agents added which will inhibit the growth of one group of organisms while permitting the growth of another. For example, Columbia CNA agar has the antibiotics colistin and nalidixic acid added which inhibit the growth of Gram-negative bacteria but not the growth of Gram-positives. It is, therefore, said to be selective for Gram-positive organisms, and would be useful in separating a mixture of Gram-positive and Gram-negative bacteria.

2. Differential media: A differential medium contains additives that cause an observable color change in the medium when a particular chemical reaction occurs. They are useful in differentiating bacteria according to some
biochemical characteristic. In other words, **they indicate whether or not a certain organism can carry out a specific biochemical reaction** during its normal metabolism. Many such media will be used in future labs to aid in the identification of microorganisms.

3. **Enrichment media:** An enrichment medium contains additives that **enhance the growth of certain organisms.** This is useful when the organism you wish to culture is present in relatively small numbers compared to the other organisms growing in the mixture.

4. **Combination selective and differential media:** A combination selective and differential medium **permits the growth of one group of organisms while inhibiting the growth of another.** In addition, it differentiates those organisms that grow based on whether they can **carry out particular chemical reactions.**

For example, **MacConkey agar (see Fig. 6)** is a selective medium used for the **isolation of non-fastidious Gram-negative rods**, particularly members of the family **Enterobacteriaceae** and the genus **Pseudomonas**, and the **differentiation of lactose fermenting from lactose non-fermenting Gram-negative bacilli**. MacConkey agar contains the dye crystal violet as well as bile salts that inhibit the growth of most Gram-positive bacteria but do not affect the growth of most Gram-negatives. If the Gram-negative bacterium ferments the sugar lactose in the medium, the acid end products lower the pH of the medium. The neutral red in the agar turns red in color once the pH drops below 6.8. As the pH drops, the neutral red is absorbed by the bacteria, causing the colonies to appear bright pink to red.

Results are interpreted as follows:

- **Strong fermentation of lactose** with high levels of acid production by the bacteria causes the colonies and confluent growth to appear **bright pink to red**. The resulting acid, at high enough concentrations, can also causes the bile salts in the medium to precipitate out of solution causing a **pink precipitate (cloudiness)** to appear in the agar surrounding the growth (see Fig. 7).
- **Weak fermentation of lactose** by the bacteria causes the colonies and confluent growth to appear **pink to red**, but without the precipitation of bile salts there is **no pink precipitate (cloudiness)** in the agar surrounding the growth (see Fig. 8).
- If the bacteria **do not ferment lactose**, the colonies and confluent growth appear **colorless** and the agar surrounding the bacteria remains relatively transparent (see Fig. 9).

Typical colony morphology on MacConkey agar is as follows:

*Escherichia coli*: colonies and confluent growth appear bright pink to red and surrounded by a pink precipitate (cloudiness) in the agar surrounding the growth (see Fig. 7).

*Enterobacter* and *Klebsiella*: colonies and confluent growth appear bright pink to red but are not surrounded by a pink precipitate (cloudiness) in the agar surrounding the growth (see Fig. 8).

*Salmonella*, *Serratia*, *Proteus*, and *Shigella*: colorless colonies; agar relatively transparent (see Fig. 9).

There are literally hundreds of special-purpose media available to the microbiologist. Today we will combine both a
mechanical isolation technique (the streak plate) with selective and selective-differential media to obtain pure cultures from a mixture of bacteria. In future labs, such as 12 - 16, which deal with the isolation and identification of pathogenic bacteria, we will use many additional special-purpose media.

MEDIA

One plate of each of the following media: Trypticase Soy agar, Columbia CNA agar, and MacConkey agar.

ORGANISMS

A broth culture containing a mixture of one of the following Gram-positive bacteria and one of the following Gram-negative bacteria:

- Possible Gram-positive bacteria:
  - *Micrococcus luteus*. A Gram-positive coccus with a tetrad or a sarcina arrangement; produces circular, convex colonies with a yellow, water-insoluble pigment on Trypticase Soy agar.
    - *Micrococcus luteus* growing on TSA
    - Close up of *Micrococcus luteus* growing on TSA
  - *Staphylococcus epidermidis*. A Gram-positive coccus with a staphylococcus arrangement; produces circular, convex, non-pigmented colonies on Trypticase Soy agar.
    - *Staphylococcus epidermidis* growing on TSA
    - Close up of *Staphylococcus epidermidis* growing on TSA

- Possible Gram-negative bacteria:
  - *Escherichia coli*. A Gram-negative bacillus; produces irregular, raised, non-pigmented colonies on Trypticase Soy agar.
    - *Escherichia coli* growing on TSA
  - *Enterobacter aerogenes*. A Gram-negative bacillus; produces irregular raised, non-pigmented, possibly mucoid colonies on Trypticase Soy agar.
    - *Enterobacter aerogenes* growing on TSA

During the next three labs you will attempt to obtain pure cultures of each organism in your mixture and determine which two bacteria you have. **Today** you will try to separate the bacteria in the mixture in order to obtain isolated colonies; **next lab** you will identify the two bacteria in your mixture and pick off single isolated colonies of each of the two bacteria in order to get a pure culture of each. The **following lab** you will prepare microscopy slides of each of the two pure cultures to determine if they are indeed pure.

PROCEDURE (to be done in pairs)

1. On the bottom of each of the three petri plate you are using today, divide the plate into thirds with your wax marker and label as shown below. This will guide your streaking.
2. Although Trypticase Soy agar (TSA), which grows both Gram-positive and Gram-negative bacteria, is not normally used as an isolation medium, we will attempt to obtain isolated colonies of the two organisms in your mixture by using strictly mechanical methods. Often, however, one bacterium overgrows another in a mixture and by the time you spread out the more abundant organism enough to get isolated colonies, the one in smaller numbers is no longer on the loop so you may not see single colonies of each on the TSA next time.

Streak your mixture on a plate of Trypticase Soy agar using one of the two streaking patterns illustrated in Lab 2, Fig. 4 and Fig. 5. agar

<table>
<thead>
<tr>
<th>Flash animation showing how to streak an agar plate for isolation: 3 sector method.</th>
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<tbody>
<tr>
<td>html5 version of animation for iPad showing how to streak an agar plate for isolation: 3 sector method.</td>
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<tr>
<td>GIF animation showing how to streak an agar plate for isolation: 5 sector method.</td>
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<tr>
<td>YouTube movie showing how to streak an agar plate for isolation: 4 sector method. Blue Ridge Community College, Virginia</td>
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3. Streak the same mixture for isolation (see Fig. 4 and Fig. 5) on a plate of Columbia CNA agar (selective for Gram-positive bacteria).

- *Micrococcus luteus* growing on Columbia CNA agar.
- *Staphylococcus epidermidis* growing on Columbia CNA agar.

4. Streak the same mixture for isolation (see Fig. 4 and Fig. 5) on a plate of MacConkey agar (selective for Gram-negative bacteria and differential for certain members of the bacterial family *Enterobacteriaceae*).

- *Escherichia coli* growing on MacConkey agar.
• *Enterobacter aerogenes* growing on MacConkey agar.

5. Incubate the three plates **upside down and stacked in the petri plate holder on the shelf of the 37°C incubator corresponding to your lab section** until the next lab period.

## RESULTS

1. Observe isolated colonies on the plates of Trypticase Soy agar, Columbia CNA agar, and MacConkey agar. Record your observations and conclusions.

<table>
<thead>
<tr>
<th></th>
<th>Trypticase Soy agar</th>
<th>Columbia CNA agar</th>
<th>MacConkey agar</th>
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<tbody>
<tr>
<td>Observations</td>
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<td>Conclusions</td>
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2. Using any of the three plates on which they are growing:

   a. **Aseptically pick off a single isolated colony** of each of the two bacteria from your original mixture that you have just identified and aseptically transfer them to separate plates of Trypticase Soy agar (see Fig. 3). Remember to **streak the plate for isolation** as you learned in labs 2 and 3.

   b. When picking off single colonies, **remove the top portion of the colony without touching the agar surface itself** to avoid picking up any inhibited bacteria from the surface of the agar. **Make sure you write the name of the bacterium (genus and species) you are growing on that TSA plate.**

   c. Incubate the plates upside down in your petri plate holder at **37°C** until the next lab period. These will be your pure cultures for Lab 5 (Direct and Indirect stains).

   Animation showing a portion of a single colony being "picked off."
PERFORMANCE OBJECTIVES FOR LAB 3

After completing this lab, the student will be able to complete the following objectives:

DISCUSSION

1. Given a mixture of a Gram-positive and a Gram-negative bacterium and plates of Columbia CNA, MacConkey, and Trypticase Soy agar, describe the steps you would take to eventually obtain pure cultures of each organism.


3. State the usefulness of Columbia CNA agar and MacConkey agar.

4. Describe how each of the following would appear when grown on MacConkey agar:
   a. *Escherichia coli*
   b. *Enterobacter aerogenes*
   c. *Salmonella*

PROCEDURE

1. Using the streak plate method of isolation, obtain isolated colonies from a mixture of microorganisms.

2. Pick off isolated colonies of microorganisms growing on a streak plate and aseptically transfer them to sterile media to obtain pure cultures.

RESULTS

1. When given a plate of Columbia CNA agar or MacConkey agar showing discrete colonies, correctly interpret the results.

SELF-QUIZ

Self-quiz

Answers

Contributors and Attributions

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