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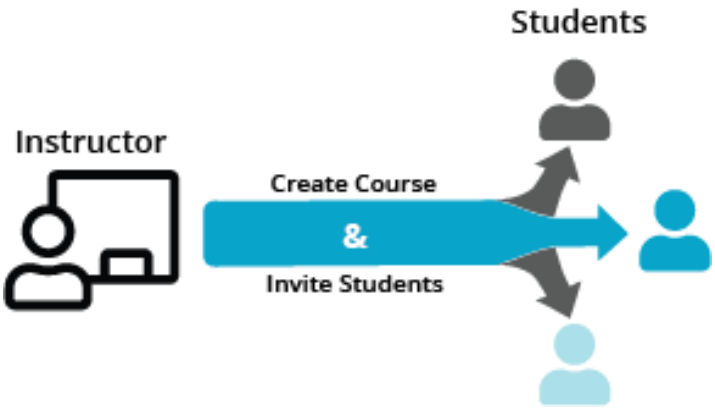

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Lab Builder

Selective and Differential Media Lab

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Instruction Level

- Undergraduate lower division (Freshman, Sophomore)
- Undergraduate upper division (Junior, Senior)

Topic

- Selective Media
- Differential Media
- Nutritive Media
- Gram-negative species
- Gram-positive species
- Staphylococcus epidermidis
- Escherichia coli
- Proteus vulgaris
- Staphylococcus aureus
- Eosin Methylene Blue agar
- Blood agar
- MacConkey agar
- Mannitol Salt
- Phenylethyl Alcohol agar

Instruction Type

- Traditional

Timeline

- **Procedure:** (Day 1) 1-2 hours
- **Incubation:** 24 hours
- **Procedure**: (Day 2) 1-2 hours
- **Data Results:** 1-2 hours

Learning Objections

- Students will learn the difference between nutritive, differential and selective media.
- Students will determine the identity of an unknown organism using different media.

Introduction

Background

Many different types of media are used in the microbiology laboratory. In general, these media can be divided into several categories, including nutritive, differential, and selective. Nutritive media are defined as media types that support the growth of a wide range of microorganisms. These types are typically considered nonselective due to the fact that they will grow most organisms. Examples of nutritive media include tryptic soy agar, nutrient agar, and blood agar.

Differential medium types are those that distinguish microorganisms from one another based on growth characteristics evident when grown on specific medium types. Organisms with differing growth characteristics typically show visible differences in growth when placed on differential media. Examples include blood agar, Eosin Methylene Blue (EMB) agar, Mannitol Salt agar, and MacConkey agar.

Selective medium types are formulated to support the growth of one group of organisms but inhibit the growth of another. These media contain antimicrobials, dyes, or alcohol to inhibit the growth of the organisms not targeted for study. Selective medium types include EMB agar, Mannitol Salt agar, MacConkey agar, and Phenylethyl Alcohol (PEA) agar.

Some media may possess both selective and differential properties, allowing them to grow certain groups of organisms of interest and give the investigator a way to discern differences in the group based on a visible reaction with the media. These media can be powerful diagnostic tools in both medical and environmental settings.

Eosin Methylene Blue agar (EMB agar)

EMB contains the dyes eosin and methylene blue. They inhibit Gram-positive organisms. Such a medium is selective for Gram-negative species. Lactose-fermenting organisms such as E. coli produce a black precipitate on EMB. Their colonies will be either black or possess dark centers with transparent, colorless peripheries. Non-lactose fermenters such as Proteus sp., Salmonella sp., or Shigella sp. appear pink or uncolored. Thus, the medium is considered differential with respect to lactose fermentation.

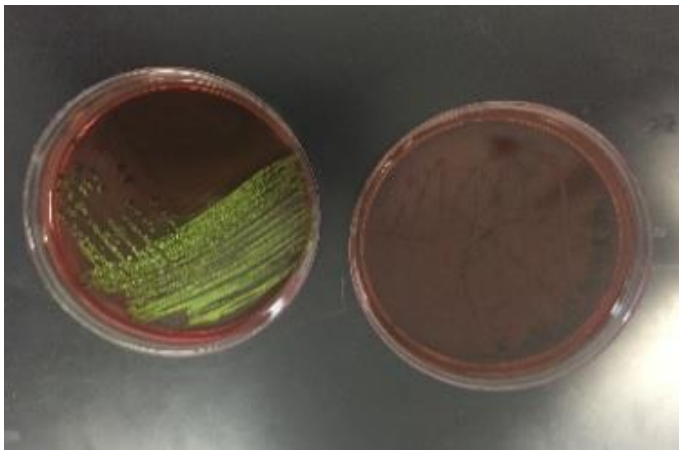

Figure 1: Eosin Methylene Blue agar

MacConkey agar

MacConkey agar is similar to EMB agar in that it is also selective for Gram-negative species and differential with respect to lactose fermentation. MacConkey agar is used for the detection of coliforms and enteric pathogens based on their ability to ferment lactose. Lactose-fermenting bacteria appear red to pink while non-lactose fermenting bacteria appear as colorless or transparent colonies.

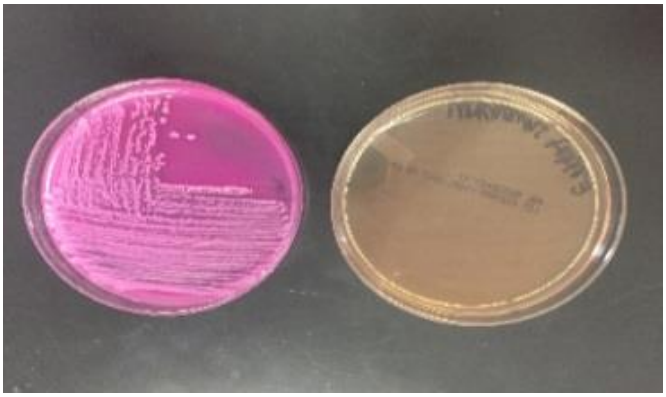

Figure 2: MacConkey Agar

Mannitol salt agar

Mannitol salt agar (7.5% NaCl) is a medium selective for staphylococci and differential with respect to mannitol fermentation. Growth of most bacteria other than staphylococci, which are halophilic, is inhibited by the high concentration of salt in the medium. Fermentation of mannitol is only seen in the pathogenic species of Staphylococcus and is signaled by the production of acidic products leading phenol red in the media to change from a neutral red-orange to bright yellow. Non-pathogenic staphylococci produce small colonies surrounded by red or pink- purple zones due to the production of basic by products due to metabolism.

Phenylethyl Alcohol (PEA)

Phenyethyl alcohol agar is a selective medium, which inhibits the growth of most Gram-negative organisms. Phenylethyl alcohol acts to disrupt lipid structure in the membrane of Gram-negative organisms as well as inhibiting protein synthesis, leading to poor growth of these organisms on the medium. The growth of Gram-negative organisms on this medium tends to be stunted or completely halted.

Blood Agar

Blood agar (5% sheep's blood- TSA plates) is a nutritive medium with differential properties in respect to hemolysis. Hemolysis is the destruction of erythrocytes (RBCs). The degree to which the erythrocytes are destroyed can be recognized by the effects of hemolytic enzymes on the cells in the medium. Complete breakdown of the RBCs is termed beta (β) hemolysis and is recognized by clearing around the colonies creating the hemolysin. Partial destruction of the RBCs leads to a greenish brown color on the agar and is termed alpha(α) hemolysis. Gamma (γ) hemolysis is usually the term applied to growth on blood agar that causes no damage to the RBCs and no change in the medium.

Pre-Lab Assignment

1. *Purpose Statement:* In one or two sentences, specifically describe the purpose of the day's experiment/lab work. What are you trying to learn or achieve, and how?
2. What is the difference between a nutritive, selective and differential media?
3. What is an example of a nutritive media?
4. What is an example of a selective media?
5. What is an example of a differential media?

Procedure

Safety

- Please refer to the biosafety guidelines for handling microorganisms in teaching laboratories.
- Wear gloves and safety goggles!

Protocol

Part 1: Selective and Differential Media

Each table group will need the following:

- 2 EMB agar plates
- 2 MacConkey agar plates
- 2 Mannitol Salt agar plates
- 2 PEA agar plates

Cultures of the following organisms will be supplied:

- *Staphylococcus epidermidis*
- *Escherichia coli*
- *Proteus vulgaris*
- *Staphylococcus aureus*
- Unknown culture

Known specimens:

1. One plate of each type should be labeled with the table number or initials of the group. Using the china pencil, draw two lines on the back of the plate to form an "X" on the back of the plate. Make sure the lines are dark enough to show through the other side. Refer to the image below.

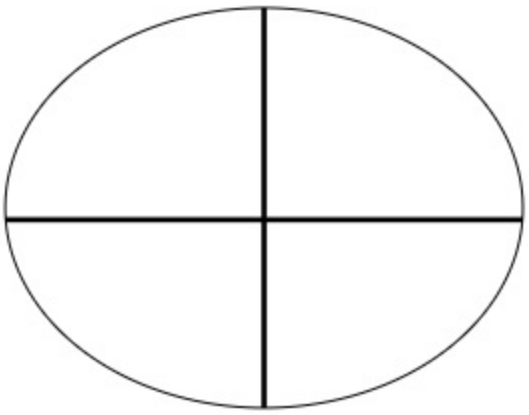

Figure 3: Preparation of agar plate

2. Label each section of the plate with the organisms made available.
3. Using aseptic technique, inoculate each plate section with the appropriate organism.
4. Continue until all plates are inoculated in the same fashion.
5. Place the plates inverted into the wire racks provided so they can be incubated.

Unknown specimen:

1. On the remaining plate of each type of medium, label as in the previous step with the table number/group name and mark the plates "unknown."
2. Using aseptic technique, inoculate each plate with the mixed culture provided.
3. Invert the plates and place them in the rack provided.

Day 2:

1. Each group will retrieve their plates and will observe the results of each of the known organisms. Record observations in table 1.
2. Then, each group will compare the known results to the unknown results to try to identify the organisms that are used in the mixed culture.
3. Students should pay close attention to the results shown by each organism and should be able to interpret the results and understand the significance of the results.

Part 2: Examination of Hemolysis

Each table group will need the following:

- 2 Blood agar plates

Cultures of the following:

- *Streptococcus pyogenes*
- *Streptococcus pneumoniae*

- *Staphylococcus aureus*
- *Staphylococcus epidermidis*

1. Using the same method as before, label each plate with the group number and name. Divide the plate into quadrants using the china pencil, drawing an"X" on the back of the plate.
2. Label each quadrant with the name of one of the organisms provided.
3. Using aseptic technique, inoculate each area with the appropriate organism.
4. Invert the plates and give them to the professor to place in a candle jar for incubation. A candle jar is a chamber filled with the oxygen evacuated to create hypoxic environment suitable for anaerobic organisms. We will utilize the candle jar again later in the semester.
5. Record observations in Table 2.

Results

Use the table to summarize your results for each organism and media type.

Table One				
	EMB	**Manitol Salt**	**MacConkey**	**PEA**
S. epidermidis				
E. coli				
P. vulgaris				
Unknown				

Use the table to summarize the results from the hemolysis experiments.

Table Two			
	Alpha	**Beta**	**Gamma**
Staph. Epidermidis			
Strep. Pyogenes			
Staph. Aureus			
Strep. pneumoniae			

Post-Lab Assignment

1. Based on your experimental results, what do you believe is the identity of the unknown?

2. Why?