Special Media for Isolating Bacteria

Objectives

After completing this exercise, you should be able to:

1. Differentiate between selective and differential media.
2. Provide an application for enrichment and selective media.

Background

One of the major limitations of dilution techniques used to isolate bacteria is that organisms present in limited amounts may be diluted out on plates filled with dominant bacteria. For example, if the culture to be isolated has 1 million of bacterium A and only 1 of bacterium B, bacterium B will probably be limited to the first sector in a streak plate. To help isolate organisms found in the minority, various enrichment and selective culturing methods are available that either enhance the growth of some organisms or inhibit the growth of other organisms. **Selective media** contain chemicals that prevent the growth of unwanted bacteria without inhibiting the growth of the desired organism. **Enrichment media,** which are usually liquid media, contain chemicals that enhance the growth of desired bacteria. Other bacteria will grow, but the growth of the desired bacteria will be increased.

Another category of media useful in identifying bacteria is **differential media.** These media contain various nutrients that allow the investigator to distinguish one bacterium from another by how they metabolize or change the media with a waste product.

Because multiple methods and multiple media exist, you must be able to match the correct procedure to the desired microbe. For example, if bacterium B is salt-tolerant, a high concentration (>5%) of salt could be added to the culture medium. Physical conditions can also be used to select for a bacterium. If bacterium B is heat-resistant, the specimen could be heated before isolation. Dyes such as phenol red, eosin, or methylene blue are sometimes included in differential media. Products of bacterial metabolism can react with these dyes to produce a color change in the medium. You will study bacterial metabolism in the exercises in Part 4. The dyes (eosin and methylene blue) in eosin methylene blue (EMB) agar are also selective. These dyes inhibit the growth of some bacteria. Three culture media will be compared in this exercise (Table 12.1).

Table 12.1

Major Chemical Components of Media Used in This Exercise

	Nutrient Agar	Mannitol Salt Agar	EMB Agar
Peptone	0.5%	1.0%	1.0%
NaCl	0.8%	7.5%	
Agar	1.5%	1.5%	1.5%
Mannitol		1.0%	
Lactose, sucrose			0.5% each
Eosin			0.04%
Methylene blue			0.0065%
Phenol red		0.025%	

Materials

Petri plates containing nutrient agar (2)

Petri plates containing mannitol salt agar (2)

Petri plates containing EMB agar (2)

Gram-staining reagents

Cultures

Escherichia coli

Micrococcus luteus

Pseudomonas aeruginosa

Staphylococcus epidermidis

Unknown mixed culture

Techniques Required

Compound light microscopy, Exercise 1

Smear preparation, Exercise 3

Gram staining, Exercise 5

Aseptic technique, Exercise 10

Inoculating loop technique, Exercise 10

Streak plate procedure, Exercise 11

Procedure

1. Using a marker, divide one nutrient agar plate into four sections by labeling the bottom. Repeat to mark one mannitol salt plate and one EMB plate. Label one quadrant on each plate for each culture.

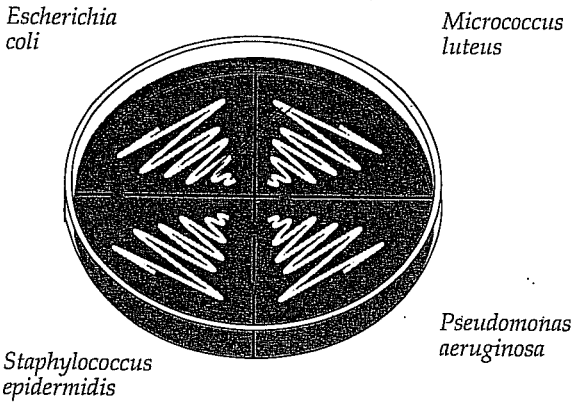


Figure 12.1

Divide a Petri plate into four sections by drawing lines on the bottom of the plate. Inoculate each section by streaking it with an inoculating loop.

2. Streak each culture on the agar, as shown in Figure 12.1.
3. Label the remaining nutrient agar, mannitol salt, and EMB plates with the number of your unknown. The unknown contains two different bacteria.
4. Streak your "unknown" onto the appropriate plates using the streaking technique shown in Figure 11.2.
5. Incubate the plates in an inverted position at 35°C. Record the results after 24–48 hours. (See Color Plates V.1, XII.1, XII.2, and XII.3.)
6. Perform a Gram stain on one colony of each different organism. Why don't you have to perform a Gram stain on each organism from every medium?

Exercise 12

Special Media for Isolating Bacteria

LABORATORY REPORT

NAME _____

DATE _____

LAB SECTION _____

Purpose

_____

_____

_____

Data

Organism	Nutrient Agar		Mannitol Salt Agar		EMB Agar		Gram-Stain Results
	Growth: +/–	Appearance	Growth: +/–	Appearance	Growth: +/–	Appearance	
E. coli							
M. luteus							
P. aeruginosa							
S. epidermidis							
Unknown							

Questions

1. What two organisms are in your mixed culture? _____

   Could you identify them from the Gram stain? _____

   How did you identify them? _____

   _____

2. How did the results observed on the mannitol salt and EMB correlate to the Gram reaction of the bacteria?

   _____

   _____

3. Which medium is selective? _____

4. What is the purpose of peptone in the media? _____

_____

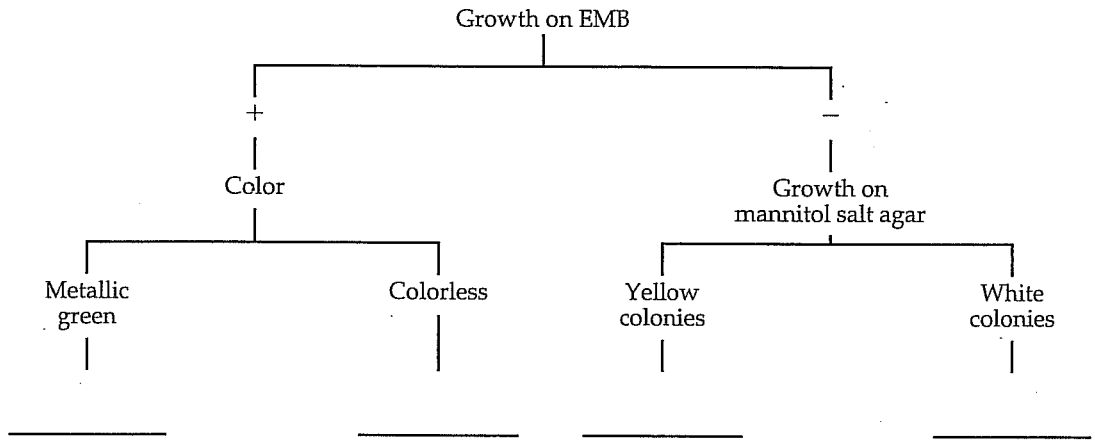
Of agar in the media? _____

_____

Critical Thinking

1. What ingredient makes mannitol salt selective? _____

2. Fill in the blanks in this diagram to make a key to these bacteria: *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*.



Circle or highlight the gram-positive bacteria in one color; use a different color to circle or highlight the gram-negative bacteria.

3. Design an enrichment medium to isolate a detergent-degrading bacterium that is found in soil.