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Advanced Laboratory Techniques

Third class

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Lecture / 4

Culture Media / part 1

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- Culture media are nutrient-rich substances used in laboratories to support the growth and propagation of microorganisms, such as bacteria, fungi, and viruses.
- These media provide the essential nutrients required for microbial growth, and they can be solid or liquid, depending on the specific needs of the microorganisms being cultivated.
- Culture media play a critical role in various fields, including microbiology, biotechnology, and medical research, by allowing scientists to isolate, cultivate, and study microorganisms.

1. **Composition:** Culture media can be classified into various categories based on their composition. These include:

- **Defined Media:** These media have precisely known compositions, with all individual components and their quantities specified. They are used when researchers want to precisely control the conditions of microbial growth.

- **Complex Media:** Complex media have more complex and undefined compositions. They are often used when cultivating microorganisms with unknown nutrient requirements because they contain a mixture of various nutrients.

- **Simple media**: It's a general-purpose media that supports the growth of non-fastidious microbes, and it is primarily used for the isolation of microorganisms. Examples are nutrient broth, peptone water, and nutrient agar.

- **Synthetic media:** Synthetic media is a type of chemically defined media and is produced from pure chemical substances. A defined media refers to a medium having a known concentration of ingredients, like sugar (glucose or glycerol) and nitrogen source (such as ammonium salt or nitrate as inorganic nitrogen). It is generally used in scientific research.

2. **Physical State:** Culture media can exist in different physical states, including:

- **Solid Media:** Agar is often used to solidify culture media. Solid media are used for purposes such as isolating and enumerating colonies of bacteria or for testing microbial susceptibility to antibiotics.

- **Semisolid media:** This media has 0.2-0.5% agar concentration, and due to the reduced agar concentration, it appears as a soft, jelly-like substance. It's mainly used to study the motility of microorganisms, distinguish between motile and non-motile bacterial strains (through U-tube and Cragie's tube), and cultivate

microaerophilic bacteria – bacteria on this media appear as a thick line. Examples of semi-solid media are: Hugh and Leifson's oxidation fermentation medium, Stuart's and Amies media, and Mannitol motility media.

- **Liquid Media:** Liquid media are typically used for the growth of microorganisms in suspension. They are well-suited for studying microbial physiology and for producing microbial biomass.

3. **Sterility:** It's crucial for culture media to be sterile to prevent contamination. Media are often autoclaved or sterilized by other means to ensure that no unwanted microorganisms are present before inoculating them with the desired microorganisms.

4. Function:

- **Basal Media:** Basal media provide essential nutrients for the growth of a wide range of microorganisms. They serve as a foundation upon which additional components can be added to create specialized media.
- **Enriched Media:** Enriched media contain extra nutrients and growth factors to support the growth of fastidious (nutritionally demanding) microorganisms.
- **Transport Media:** These media are used for the transportation of clinical specimens containing microorganisms from the patient to the laboratory. They aim to keep the microorganisms viable during transit.
- **Storage Media:** Storage media are designed to maintain the viability of cultures for extended periods, often by reducing metabolic activity and preserving the organisms
- **Differential Media:** Differential media are used to distinguish between different types of microorganisms based on their growth characteristics or metabolic activities. They often contain indicators, such as dyes, which change color in response to specific metabolic activities.\
- **Selective Media:** These media are designed to encourage the growth of specific microorganisms while inhibiting the growth of others. They typically contain substances that favor the growth of certain species and suppress the growth of unwanted ones.

5. Use or Application:

- **Clinical Media:** Media used in clinical microbiology labs for diagnosing and identifying pathogens.
- Industrial Media: Media used in industrial processes like fermentation for the production of various products, such as antibiotics and enzymes.
- **Research Media:** Media used in scientific research to study microbial physiology, genetics, and other aspects of microorganisms.

- **Diagnostic Media:** Media used for specific diagnostic tests, such as antibiotic susceptibility testing or identification of particular pathogens.

6. Special Characteristics:

- **Selectivity and Differential**: These media combine both selective and differential properties to isolate and identify specific microorganisms.
- **Indicator Media**: These media contain indicators that change color in response to specific metabolic activities, aiding in the identification of microorganisms.

7. pH and Chemical Properties:

Some media may be classified based on their pH (e.g., acidic, alkaline) or specific chemical properties (e.g., media designed for anaerobic growth).

a. Microorganism Growth Requirements:

- **Aerobic Media**: Promotes the growth of microorganisms that require oxygen.
- **Anaerobic Media**: Facilitates the growth of microorganisms that grow in the absence of oxygen.
- **Facultative Anaerobic Media**: Supports the growth of microorganisms that can grow with or without oxygen.

b. Temperature Range:

- **Psychrophilic Media**: For organisms that grow at low temperatures.
- **Mesophilic Media**: For organisms that grow at moderate temperatures.
- **Thermophilic Media**: For organisms that thrive at high temperatures.

c. pH Range:

- **Acidic Media**: For acidophilic microorganisms that grow well in acidic conditions.
- **Alkaline Media**: For alkaliphilic microorganisms that prefer alkaline conditions.
- **Neutral Media**: Maintains a neutral pH suitable for most microorganisms.

8. <u>Aseptic technique</u>

- Aseptic technique is a set of practices and procedures used to prevent the contamination of microbial cultures, substances, and the environment with unwanted microorganisms, including bacteria.
- It is commonly employed in microbiology, clinical laboratories, and various other scientific and industrial settings where the purity and integrity of microbial cultures are crucial.

- When transferring bacteria using aseptic technique, the primary goals are to minimize the risk of contamination and ensure that the culture remains pure and viable.
- There's how it's typically done:

A. Wash Hands: Start by thoroughly washing your hands with soap and water. This step is crucial to remove any potential contaminants from your hands.

B. Prepare Your Workspace:

- Disinfect the work area, such as the lab bench or laminar flow hood, with a suitable disinfectant like ethanol or a bleach solution.
- Place all necessary tools, such as inoculating loops, pipettes, and culture plates, within your reach. Make sure they have been sterilized.

C. Flame Sterilization:

- If using an inoculating loop or wire, heat it over a Bunsen burner or alcohol lamp flame until it glows red. This sterilizes the loop.
- Allow the loop to cool briefly, so it doesn't harm the bacteria when you touch the culture.

D. Opening the Culture:

- With one hand, gently lift the lid or cap of the culture vessel (e.g., a Petri dish or test tube). Keep the other hand free for handling the loop.
- Avoid breathing directly over the culture to prevent contamination from airborne microorganisms.

E. Transfer the Bacteria:

- Insert the sterilized loop or pipette tip into the culture.
- If you're transferring bacteria from a solid medium (like an agar plate), gently touch the surface of the bacterial colony with the loop to pick up a small amount of the culture.
- If you're transferring from a liquid medium (broth), insert the pipette tip into the culture and draw up the desired volume.
- Replace the lid or cap on the culture vessel to minimize exposure to contaminants.

F. Streaking or Inoculating:

- For solid media, streak the loop back and forth on the target medium (agar plate) to create a streak pattern or inoculate according to the desired method.
- For liquid media, release the contents of the pipette into the new culture vessel.

G. Flame Sterilization Again:

- After transferring the bacteria, re-sterilize the inoculating loop or pipette tip by passing it through the flame to prevent carryover contamination.

H. Close and Label:

- Seal the culture vessel with its lid or cap.
- Label the new culture with essential information like the date, bacterial strain, and any specific details regarding the transfer.

I. Dispose of Waste Properly:

- Dispose of used inoculating loops or pipette tips in a designated container for contaminated materials.
- Dispose of any contaminated materials properly to avoid spreading potential contaminants.

- Aseptic technique is essential to maintain the purity and integrity of bacterial cultures and to ensure accurate experimental results and diagnoses in microbiology and related fields.
- It reduces the risk of cross-contamination and the introduction of unwanted microorganisms.

9. Bacterial colony characteristics

- Bacterial colony characteristics refer to the visible attributes and features of bacterial colonies that develop on solid agar media.
- These characteristics are used in microbiology for the identification and classification of bacterial species.
- Several colony characteristics are considered when describing and identifying bacterial colonies:

a) **Size**: The size of a bacterial colony can vary significantly and is typically described as small, medium, or large. This characteristic can provide initial information about the growth rate and abundance of the bacterium.

b) **Shape**: Bacterial colonies can have various shapes, including circular, irregular, filamentous, rhizoid (root-like), or punctiform (very tiny).

c) **Margin or Edge**: The edge of a bacterial colony may exhibit characteristics such as smooth, undulate (wavy), lobate (lobe-like), filamentous, or curled. The margin can provide clues about the colony's growth pattern.

d) **Elevation**: The elevation of a colony refers to its height above the agar surface. Common elevation descriptors include flat, raised, convex, pulvinate (cushion-shaped), or umbonate (having a raised central region).

e) **Color**: The color of a bacterial colony can vary and may be described using terms like white, cream, yellow, pink, red, orange, or transparent. The colony color can result from the production of pigments by the bacteria.

f) **Opacity or Transparency**: Bacterial colonies can be opaque (not see-through) or transparent (see-through). The degree of transparency can be important in identifying certain bacterial species.

g) **Texture**: Colony texture can be smooth, mucoid (slimy or glistening), rough, or wrinkled. Mucoid colonies often result from the production of a polysaccharide capsule.

h) **Surface Topography**: The surface of a colony can have different characteristics, such as shiny, matte (dull), or granular.

i) **Consistency**: The consistency of a bacterial colony may be described as dry, moist, or mucoid. The presence of moisture or mucus can be indicative of specific bacterial characteristics.

j) **Hemolysis**: In the case of blood agar plates, the ability of bacteria to break down red blood cells in the agar can result in different patterns. This can be described as alpha-hemolysis (greenish discoloration around the colony), beta-hemolysis (complete clearing of the blood agar), or gamma-hemolysis (no hemolysis).

k) **Odor**: In some cases, bacterial colonies may have characteristic odors, which can be a useful diagnostic feature. Odors can range from sweet to putrid.

l) **Growth Rate**: The time it takes for a colony to reach a specific size can provide information about the bacterium's growth rate. Bacterial colonies can be classified as fastidious (slow growers) or rapid growers.

m) **Gram Staining**: Although this is not an attribute of the colony itself, it is often performed in conjunction with colony characterization. Bacterial colonies can be

classified based on their Gram stain characteristics, as Gram-positive or Gram-negative.

- These colony characteristics are observed and recorded as part of the process of identifying and characterizing bacterial species in microbiology. By analyzing these features, microbiologists can make initial determinations about the potential identity and characteristics of the isolated bacteria.
- Macroscopic examination of bacterial cultures involves the visual observation of bacterial colonies on solid agar media. This examination is a fundamental step in the identification and characterization of bacteria in microbiology.

10. **Gram staining**

- Gram staining is a widely used microbiological technique that allows for the differentiation of bacterial cells into two major groups: Gram-positive and Gram-negative based on the characteristics of their cell walls.
- The method was developed by Hans Christian Gram in the 1880s.
- there is a step-by-step guide on how to perform Gram staining:

Materials and Reagents:

1. Bacterial culture
2. Microscope slides
3. Bunsen burner or alcohol lamp
4. Inoculating loop or sterile wooden stick
5. Crystal violet stain
6. Gram's iodine (iodine-potassium iodide) solution
7. Ethanol or isopropanol (alcohol)
8. Safranin or basic fuchsin stain
9. Water or ethanol for washing
10. Microscope

Procedure:

1. Prepare a bacterial smear:

- Place a drop of water on a clean microscope slide.
- Aseptically transfer a small amount of your bacterial culture onto the slide using an inoculating loop or sterile wooden stick.
- Spread the bacterial culture evenly on the slide and let it air-dry. Be gentle to avoid damaging the cells.

2. Heat-fix the smear:

- Pass the slide (smear side up) through the flame of a Bunsen burner or alcohol lamp a few times to heat-fix the bacteria. This helps to attach the bacterial cells to the

slide and kills the bacteria, preventing them from washing away during the staining process.

3. Stain with crystal violet:

- Cover the bacterial smear with crystal violet stain and let it sit for about 1 minute.
- Rinse the stain off with water.

4. Iodine treatment:

- Flood the slide with Gram's iodine solution for about 1 minute. This step serves to fix the crystal violet stain in the bacterial cells.
- Rinse the slide with water.

5. Decolorization:

- Gently tilt the slide and add ethanol (or isopropanol) drop by drop until no more color washes off (usually for about 10-20 seconds). This step is crucial as it differentiates between Gram-positive and Gram-negative bacteria.

6. Counterstain with safranin:

- Cover the smear with safranin or basic fuchsin stain and let it sit for about 1-2 minutes.
- Rinse off the stain with water.

7. Blot dry:

- Gently blot the slide with blotting paper or a paper towel to remove excess water.

8. Examine under the microscope:

- Place the slide on the stage of a light microscope.
- Examine the stained bacterial cells using oil immersion (100x objective) to visualize the cell morphology and staining characteristics.

Interpretation:

- **Gram-positive bacteria** retain the crystal violet stain and appear purple under the microscope.
- **Gram-negative bacteria** lose the crystal violet stain during decolorization and take up the safranin or basic fuchsin, appearing pink or red under the microscope.