Tutorial session 4: Culture media

I. Introduction

The discovery of culture media has enabled a transition from simple microscopic examination to bacterial isolation on media that support their growth, followed by biochemical identification and antibiotic susceptibility testing.

II. Definition and basic composition of culture media

II.1. Definition

A culture medium is a microbiological preparation that provides essential nutrients in adequate amounts to support bacterial metabolism, survival, and multiplication. Additionally, it must have appropriate physicochemical properties—such as pH, isotonicity, and redox potential—to create optimal conditions for bacterial growth.

II.2. Composition

A culture medium is composed of essential elements, including water, mineral salts, and nutrients that serve as structural and energy sources. It may also contain a pH or redox reaction indicator to monitor metabolic activity or differentiate bacterial groups.

The composition of a culture medium varies depending on the objective and the specific requirements of the bacteria. A basic culture medium must include:

- A water source (distilled water).
- A carbon or energy source (e.g., glucose).
- A nitrogen source (e.g., ammonium salts or amino acids) and a sulfur source.
- A phosphorus (P) and potassium (K) source.
- A calcium (Ca) and magnesium (Mg) source, essential for enzyme activity and membrane stability.
- Trace elements (e.g., iron, copper, zinc, cobalt salts) necessary for enzymatic functions.
- A pH buffer to maintain neutrality, along with a pH or redox reaction indicator to track microbial metabolism.

Additionally, growth factors and vitamins may be added to support the growth of auxotrophic bacteria that require specific nutrients for survival.

III. Classification of culture media

There are various criteria for classifying culture media.

III.1. Classification according to the composition

- **Complex (empirical/natural) media:** Their exact chemical composition is not fully defined, as they provide nutrients in an unstandardized form, resembling the bacteria's natural environment. They typically contain meat extracts, yeast extracts, and peptones.

Example: Brain Heart Infusion Broth (BHIB).

- **Semi-synthetic media**: These are complex media supplemented with chemically defined substances, such as growth factors, blood, or vitamins, to support the growth of fastidious bacteria. Example: Sheep blood-enriched agar.
- **Synthetic media**: Their composition is precisely known in both quantity and quality. They are used for enzymatic reaction studies or to investigate the specific nutritional needs of microorganisms. **Example:** Ferguson medium.

III.2. Classification according to the consistency

- **Liquid media**: Used for the growth and enrichment of bacteria in suspension.

Example: Clark and Lubs medium, enrichment broth.

- **Solid (agar-based) agar-based media**: These are liquid media solidified with agar (1–1.7% concentration). Agar is a polysaccharide derived from red algae, which solidifies (forming a solid gel) at around 42°C.

Example: Chapman medium, TSI, Hektoen medium, PCA medium.

- **Semi-liquid or semi-solid media**: These contain a lower agar concentration (0.05–0.075%) and are often used for motility testing or anaerobic growth.

Example: Mannitol motility medium, MEVAG medium.

III.3. Classification according to the usage

- **General-purpose (basic) media**: Support the growth of non-fastidious bacteria.

Example: Nutrient agar and nutrient broth.

- **Enriched media**: Contain additional nutrients or growth factors essential for fastidious bacteria.

Example: Blood agar, vitamin-supplemented agar.

- **Enrichment media**: Liquid media that selectively enhance the growth of a target bacterium from a mixed sample, particularly when bacterial counts are low (paucibacillary samples).

Example: Blood culture broth, Buffered Glucose Broth (BGT).

- **Selective media**: Promote the growth of a specific bacterial type by using inhibitory substances to suppress others. These inhibitors vary and may include:
  - NaCl: Chapman medium (75‰) for *Staphylococcus*, hypersaline broth (65‰) for *Enterococcus*.
  - Bile salts (deoxycholate): MacConkey and Hektoen media for *Enterobacteriaceae*.
  - Antibiotics: Blood agar + ANC (nalidixic acid, colistin) for *Streptococcus*.
  - Antiseptics: Cetrimide agar for *Pseudomonas*.

Note: Selectivity can also be increased by adjusting culture conditions, e.g., incubating cetrimide agar at 42°C enhances selectivity for *Pseudomonas aeruginosa*.

- **Elective media**: Encourage the predominant growth of a specific bacterial genus while allowing other bacteria to grow.

Example: Coagulated serum agar for *Corynebacteria.*

- **Identification media**: Used to study bacterial biochemical metabolism.

Example: TSI (Triple Sugar Iron), Ferguson medium.

- **Preservation media**: Used to maintain bacterial viability.
  - Non-fastidious bacteria: Stored on deep agar in capillary tubes at room temperature.
  - Fastidious bacteria: Stored in liquid media (BGT + glycerol) and frozen at -80°C.

- **Transport media**: Maintain bacterial viability during transport, varying based on bacterial fragility.

Example: TGV (Transport Medium for Live Germs), charcoal medium for fragile bacteria.

- **Antibiogram media**: Used for antibiotic susceptibility testing, designed to mimic biological fluids to ensure in vitro antibiotic activity correlates with in vivo results.

Example: Mueller-Hinton agar

- **Chromogenic (differential) media**: Facilitate preliminary bacterial identification through enzyme-specific colour reactions. These media incorporate colourless substrates that bacterial enzymes convert into coloured colonies.

Example: Uriselect which contains urea for urease detection (produced by *Proteus*).
Note: Some chromogenic media also detect specific antibiotic resistance mechanisms by incorporating targeted antibiotics.
Example: Detection of Extended-Spectrum Beta-Lactamase (ESBL) or Methicillin-Resistant *Staphylococcus aureus* (MRSA).

III.4. Classification according to the sterilization method

There are two types of culture media:
a) **Autoclavable media**: Culture media whose components are not destroyed by heat. *Example: Nutrient agar, Mueller-Hinton broth in bottles.*
b) **Non-autoclavable media**: Culture media containing heat-sensitive (thermolabile) components such as proteins, vitamins, or some antibiotics, which can be degraded by heat. Instead of autoclaving, they are sterilized using filtration.
Example: Löwenstein-Jensen medium.

III.5. Classification according to the commercial presentation

a) **Dehydrated media**: These come in powder form, typically packaged in 250g or 500g containers.
b) **Ready-to-use media**: Several forms of ready-to-use media, whether solid or liquid, are commonly encountered or used, including:

- Agar in bottles
- Agar in Petri dishes
- Slant agar in tubes (e.g., Simmons citrate agar)
- Butt and slant agar (e.g., TSI)
- Deep agar (e.g., MEVAG)
- Deep agar in capillary tubes (e.g., VF – meat-liver medium, used for preservation)
- Liquid media in bottles (e.g., blood culture broth)
- Liquid media in tubes (e.g., BGT – Buffered Glucose Broth)
- Liquid media in ampoules (e.g., AA – Amino Acids medium)

The choice of form depends on the type of microbiological analysis and the specific requirements of the microorganisms to be cultivated.