

Lec.4 cultivation of bacteria in culture media

➤ Culture media:

Definition : It is the media containing the required nutrients for bacterial growth.

Uses: 1- Isolation and identification of micro-organisms
2- Performing anti-microbial sensitivity tests

➤ Common ingredients of culture media

1- Peptone: Hydrolyzed product of animal and plant proteins: Free amino acids, peptides and proteoses (large sized peptides).

** It provides nitrogen; as well carbohydrates, nucleic acid fractions, minerals and vitamins.

2- Meat extract: supply amino acids, vitamins and mineral salts.

3- Yeast extract: It is bacterial growth stimulants.

4- Mineral salts: these are: 1- Sulfates as a source of sulfur.

2- Phosphates as a source of phosphorus.

3- Sodium chloride & Other elements

5- Carbohydrates: Simple and complex sugars are a source of carbon and energy.

** Assist in the differentiation of bacteria :

Ex.- Sucrose in TCBS agar differentiates vibrio species.

-Lactose in MacConkey agar differentiates enterobacteria.

6- Agar: It is an inert polysaccharide of seaweed, It is not metabolized by micro-organism.

Property:

1. It has high gelling strength
2. high melting temperature(90-95 C⁰)
3. low gelling temperature
4. It forms firm gel at 1.5% W/V concentration.
5. It forms semisolid gel at 0.4-0.5% W/V concentration.

Uses:

1- Solidify culture media

2- May provide calcium and organic ions to inoculated bacteria.

7- Water : Deionized or distilled water must be used in the preparation of culture media.

➤ Types of culture media:

1. Basic /Simple / All purpose media

It is a media that supports the growth of micro-organisms that do not require special nutrients.

Uses :

1. To prepare enriched media
2. To maintain stock cultures of control bacterial strains
3. To subculture pathogenic bacteria from selective/differential medium prior to performing biochemical or serological tests.

Example : Nutrient Broth , Nutrient Agar.

2. Enriched media

Media that are enriched with whole blood, lyzed blood, serum, special extracts or vitamins to support the growth of pathogenic bacteria.

Example : Blood Agar , Chocolate Agar

Fluid media that increases the numbers of a pathogen by containing enrichments and/or substances that discourage the multiplication of unwanted bacteria.

Example : Selenite F broth media , Alkaline peptone water

4. Selective media

Media which contain substances (Eg. Antibiotics) that prevent or slow down the growth of bacteria other than pathogens for which the media are intended.

Example : Modified Thayer –Martin Agar , Salmonella-Shigella(SS) agar

A- Differential media

Media to which indicator substances are added to differentiate bacteria.

Example : TCBS Agar differentiates sucrose fermenting yellow colonies of *Vibrio cholerae* to non-sucrose fermenting blue colonies other *Vibrio* species.

NB: Most differential media distinguish between bacteria by an indicator which changes color when acid is produced following carbohydrate fermentation.

B- Transport media

Media containing ingredients to prevent the overgrowth of commensals and ensure the survival of pathogenic bacteria when specimens cannot be cultured soon after collection.

Example : Amies transport media , Stuart media , Kelly-Blair media

➤ Choice of culture media:

The selection culture media will depend on:

1. The major pathogens to be isolated, their growth requirements and the features by which they are recognized.
2. Whether the specimens being cultured are from sterile sites or from sites having normal microbial flora.
3. The cost, availability and stability of media.
4. The training and experience of laboratory staff in preparing, using and controlling culture media.

➤ Forms of culture media

1. solid culture media
2. semisolid culture media
3. Fluid culture media

1. solid culture media

- . Plate cultures in petri dishes
- . stab/slope cultures in tubes and bottles

Uses: Description of bacterial colonies

- size : diameter in mm
- Out line : circular, entire, wavy, indented

- Elevation: flat, raised, low convex and dome shaped.
- Transparency: transparent, opaque, and translucent.
- Surface: smooth (mucoid) and shiny, rough and dull.
- Color: colorless, white, pink, and pigmented
- changes in medium Eg. Hemolysis in Blood Agar , Blackening of medium due to hydrogen sulfide production.

2. Semisolid culture media

- Uses:** 1. as an enrichment media
2. as motility media

3. Fluid culture media

Bacterial growth in fluid media is shown by a turbidity in the medium.

Uses :

1. as an enrichment media
2. as biochemical testing media
3. as blood culture media

➤ **Preparation of culture media:**

Culture media contains essential ingredients for microbial growth requirements. For successful isolation of pathogens, culture media must be prepared carefully. Most culture media are available commercially in ready –made dehydrated form.

The major processes during preparation of culture media

- Weighing and dissolving of culture media ingredients
- Sterilization and sterility testing
- Addition of heat-sensitive ingredients
- Dispensing of culture media
- pH testing of culture media
- Quality assurance of culture media
- Storage of culture media

▪ **Sterilization and sterility testing**

Always sterilize a medium at the correct temperature and for the correct length of time as instructed in the method of preparation.

Methods used to sterilize culture media:

A) Autoclaving

Autoclaving is used to sterilize most agar and fluid culture media.

B) Steaming at 100 °C

It is used to sterilize media containing ingredients that would be inactivated at temperature over 100 °C and re-melt previously bottled sterile agar media.

C) Filtration

It is used to sterilize additives that are heat-sensitive and cannot be autoclaved.