

Culture Medium

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I.Culture Media Definition-

The nutrient preparation on or in which a culture(microorganisms) is grown in the laboratory is known as culture medium or culture media.

- A culture medium is a solid or liquid preparation utilized to grow, transport, and store microorganisms. To be useful, the medium must hold all the nutrients the microorganism needs for germination.
- All the microorganisms demand basic requirements, although there is a variation as to the application of organic and inorganic compounds.
- Depending upon the species to be grown, culture media vary in form and composition.
- Some media carry a solution of inorganic salts and may be supplied with one or more organic compounds while other media contains miscellaneous components such as extracts or digests of plant and animal tissues.
- Many of the studies of Microbiology are based on the capacity to grow and maintain microorganisms in the

laboratory. And this is achievable only if proper culture media are convenient.

- The selection of a suitable culture medium further depends on the information about the microorganism's natural territory because its nutritional demands reveal its natural surroundings.
- A satisfactory medium contains energy sources, carbon, nitrogen, phosphorus, sulfur and various minerals, etc. which is required for a microorganism to be grown.
- During this preparation a culture medium for any microorganisms, the main intention is to implement a stable mixture of the expected nutrients at concentrations that will allow immeasurable growth.
- Additionally, the culturing of microorganisms needs the precise control of different environmental agents which usually are kept within narrow boundaries.

2. Classification of Culture Media/Type of Culture Medium –

Many special-purpose media are required to promote the identification, enumeration, and isolation of specific types of bacteria. To satisfy these requirements, the microbiologists have prepared numerous media, on the basis of their purpose or function, composition may be classified as follows:

Classification of Culture Medium based on physical state

Based on physical state the culture medium is divided into three classes, such as;

Liquid Medium/Broth

- When only nutrients are dissolved in a solvent without any solidifying agent, then these types of media are known as liquid media or Broth.
- They are also called broth, used for the growth and production of microbes.
- The germination of cells becomes apparent in the form of a minute size on the tip of the broth.
- Mostly bacteria and fungi are cultured in liquid broth.
- Some examples of liquid broth are Nutrient broth, Glucose broth, Beef extract, Skimmed milk, Peptone solution.
- Beef extract (a beef derivative which is a reservoir of organic carbon, nitrogen, vitamins, and inorganic salts) and peptone solution (a semi-digested proteins are considered as the most widely used liquid medium in bacteriological laboratory).

Solid Medium

- The solid media may either constantly remain solid such as potato slices, coagulated blood serum, coagulated egg or can be melted for example nutrient-agar medium, nutrient-gelatin medium, potato dextrose agar medium.
- They are also known as agar medium
- Solid Medium contains 1.5%-2.0% solidifying agents such as agar with other nutrients.
- Other solidifying agents are also used in a solid medium such as silica gel, gelatin (10-20%), etc.
- These types of media are accepted for composing agar slants or slopes and agar stab.

- They are used for colony identification, colony characterization, isolation and enumeration of microbial cells, and antibiotics sensitivity tests.
- Agar is a heterogeneous polysaccharide (carbohydrate) composed of 3,6 anhydro-L-galactose, and D-galactopyranose, free of nitrogen, produced from various red algae belongs to genera Gelidium, Gracilaria, Gigartina and Pterocladia.
- Agar melts on warming to 96 C and solidifies within a jelly on reducing to 40-45 C.
- The hardened medium is deposited in a petri dish which produces an artificial habitat proper for a speedy growth of fungi.
- When in the liquefied phase, solid media can be exerted in test tubes, which are either left to cool and solidify in a slanted position agar stands, or left to freeze in an upward manner in agar deep tube.

Semi-solid Medium

- These types of media remain semi-solid condition.
- It is prepared by adding half quantity of agar (1/2 than needed for solid medium) i.e. approximately 0.5% in the medium.
- Semi-solid medium is mainly used for the cultivation of microaerophilic bacteria and the study of bacterial motility.
- This type of medium can be selective or differential media.
- The selective media encourage the growth of one organism and hinder the growth of the other organism while

differential media help to distinguish organisms growing collectively.

- An example of semi-solid medium is Cystine trypticase-agar medium.

Classification of Culture Media based on Chemical Nature

Based on the chemical nature the culture media is divided into four classes such as;

Non-synthetic Media or complex medium

- The medium that contains ingredients of unknown composition is known as Non-synthetic media, Undefined medium, or Complex medium.
- It is composed of sugar, yeast, beef extract, vitamins, peptones, salts, etc.
- Some examples of non-synthetic media are Potato-Dextrose Agar (GM-25), Solid- Extract Agar (SM-I), Oatmeal-Agar (GM-24), Malt- Extract Agar (GM-I9b), Waksman's medium (GM-40).

Synthetic media or Defined Media

- The medium which contains some ingredients of known chemical composition is known as synthetic medium.
- Synthetic Media are valuable for nutritional and metabolic experiments.
- Example: An example of a synthetic media is E. coli broth, Czapek Dox media (GM-9), and Richard's solution (GM-27).

Semi-synthetic Media

- The medium that contains some known and some unknown chemical ingredients is known as Semi-Synthetic Medium.
- An example of Semi-Synthetic Medium is Tryptic soy sugar [Trypticase (15g), Soya peptone (5g), NaCl (5g), Distilled water (1L), Ph (7.1)].

Natural Medium/ Empirical

- When a natural product is used for growing bacteria, this type of natural product is known as a natural medium.
- Some examples of natural medium are Milk, Wine, Blood, Vegetable juices, Yeast extract, Egg, etc.
- Natural media are usually very useful and support sporulation in fungi that may contrarily remain sterile.
- An example of Natural media /Empirical is Blood Agar [Infusion from beef heart(500g), Tryptone(10g), NaCl(5g), Agar(15g), Distilled water(1000ml), pH(7.3)]
- Some natural media may be made of a synthetic medium expanded by tomato juice, carrot strips of plant stems.

Classification of Medium Based on Purpose of Use

Based on the Purpose of Uses, the culture media is classified into the following groups.

Selective Media

- The selective medium inhibits the growth of the undesired microorganisms while grants and encourages the growth of desired microorganisms to develop characteristic colonies.

- The medium shows selective action due to the presence of certain chemicals to it. For example, the addition of crystal violet stain in the culture medium selectively hinders the germination of gram-positive bacteria and grants and encourages the germination of gram-negative bacteria.
- Some examples of selective medium are MacConkey Agar for *E. coli*, Deoxycholate-Citrate-Agar (DCA) for *Salmonella*, and Mannitol-salt-Agar medium for pathogenic *Staphylococci*.

The selective Media is prepared by incorporation of-

- ✓ **A specific Nature:** Cellulose as carbon source.
- ✓ **Antibiotic:** Antibacterial and antifungal antibiotics inhibit the respective groups.
- ✓ **Dye:** Rose-bengal is a bacteriostatic chemical that is added to prevent the growth of bacteria.
- ✓ **Adjusting the pH value:** The growth of specific microorganisms is supported and the growth of the others is hindered by adjusting the pH.

Differentia Medium or Indicator Media

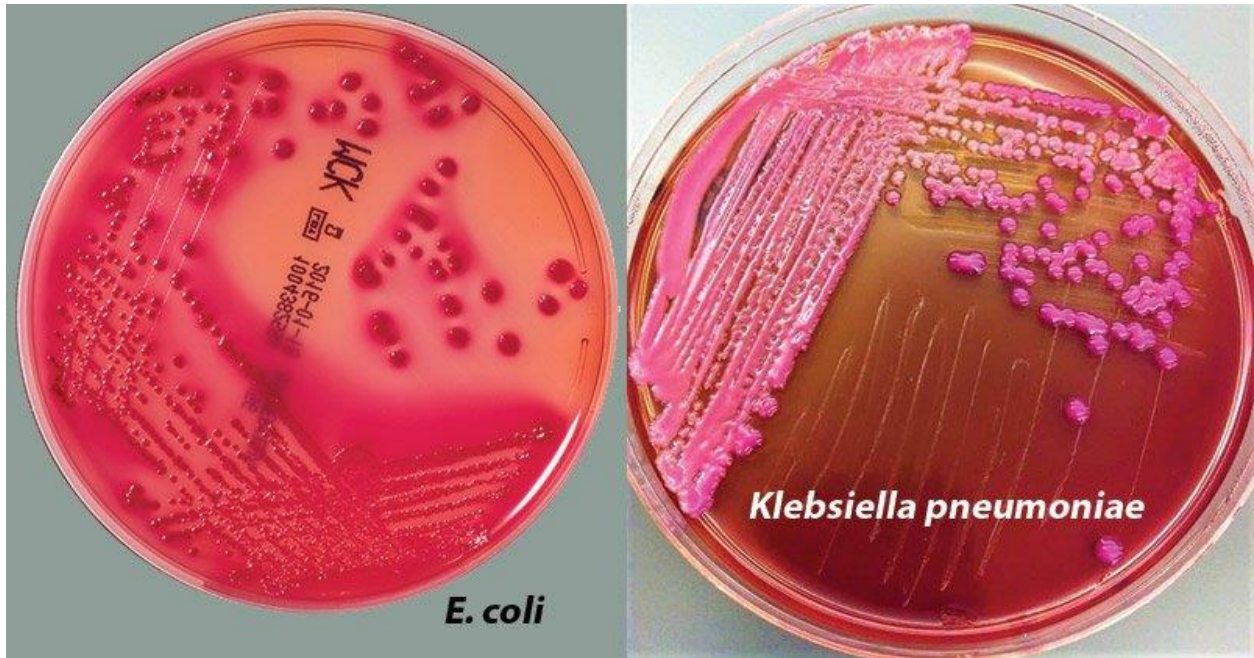
- A differentia (indicator) medium is one of those that make a noticeable difference between various groups of bacteria breeding in the medium and also allow experimental classification of microorganisms based on their biological properties.
- In one word, a medium which contains certain reagents or supplements which allows differentiation of various kinds

of microorganisms on the basis of visual differences in their growth pattern.

- Some examples of differential medium are Blood Agar Medium, MacConkey Agar Medium, and Christensen's Medium.
- Urease producing bacteria (e.g. Proteus, Klebsiella) can be identified by using Christensen's medium. This medium contains urea and phenol red, hence urease-producing bacteria break down the urea into ammonia and CO₂. Ammonia converts the medium to alkaline and in alkaline pH the medium produces pink color because phenol red shifts in red color in alkaline medium.

Enriched Medium

- Enriched media contains specific growth substances such as blood, serum, and egg with the nutrient agar to satisfy the nutritional stresses of metabolically fastidious microorganisms.
- Some examples of enriched Medium are blood agar for Haemophilus, Bardet-Gengou for isolation of Bordetella, and Loeffler's serum slope for the isolation of Corynebacterium diphtheria.
- Liquid media used to isolate pathogens from a mixed culture.
- Stimulate growth of desired bacterium inhibit growth of unwanted bacterium.
- Media is incorporated with inhibitory substances to suppress the unwanted organism → Increase in numbers of diseased bacteria.



Selective Medium (MacConkey Agar)

Hemolysis on Blood agar



beta-hemolysis
Streptococcus pyogenes

alpha hemolysis
Escherichia coli

gamma hemolysis (no hemolysis)
Staphylococcus epidermidis

✚ Assay Medium

- Media of designated composition are utilized for the assay of vitamins, amino acid, and antibiotics.
- In the medium, the rate of growth is directly proportional to the nutrient concentration.
- Biotin assay medium, used for vitamin assay, seed agar medium used for antibiotics assay.

Enumeration Medium

- Specific type of medium are used to determine the microbial content of such materials as milk (lactose agar), soil extract agar, and sewage water (lactose broth) to favor the type of organism found this material.

Biochemical Medium

- This medium is utilized to distinguish between two microorganisms on the basis of their biochemical activities.
- This type of media is effective in the study of metabolic processes and the classification of microorganisms.
- Some examples of biochemical medium are peptone broth used for testing for the production of hydrogen sulfide(H_2S) by lead acetate, that terms black.

Maintenance Medium

- Maintenance Medium is used for maintenance of viability and physiological characteristic of a culture requiring a medium in which a large number of microbes can be grown and used for routine cultivation and maintenance of a wide variety of organisms.
- Example: Fogg's Medium use for Algae, potatoes dextrose agar used for fungi.

Transport Medium

- In the medical field, transport medium is used for different purpose.
- During the transport of pathogenic microorganisms from the hospital to the laboratory transport medium are used to maintain them.
- This medium ideally maintains the viability of all organisms in the specimen without altering their concentration.
- These type of medium only contain buffers and salts.
- When the pathological laboratory is far away from the patient, the delicate pathogenic microorganism (e.g.- *Neisseria gonorrhoeae* that causes gonorrhoea) may not last, or the normal microorganisms (e.g.- *Escherichia coli*) may over grow pathogenic microorganisms (e.g.- *Salmonella*, *Shigella*, *Vibrio cholerae*) even ere the transportation of clinical sample to the tasting laboratory. To evade this, culture media have been devised to sustain viability of the pathogen.
- Example-
 - ✓ **Stuart's Medium:** Stuart's Medium utilized to keep the viability of the gonococci bacteria.
 - ✓ **Pike's medium:** pike's medium is utilized to store *streptococcus pyogenes*.
 - ✓ **Glycerol-saline Medium:** Glycerol-saline Medium is utilized to inhibit normal intestinal microflora from overgrowing the enteric fever bacilli.

✓ **Bile-Peptide Medium:** Bile-Peptide Medium is utilized for sustaining the viability of cholera causing bacteria.

✓ **Venkatraman Ramakrishnan Medium:** V.R Medium is used for transport of stools of cholera patients.

✚ **Characterization Medium**

- This medium is utilized to determine or characterize the type of growth produced by bacteria as well as to determine their ability to produce certain chemical changes.
- Gelatin stab is used for gelatin liquefaction test.

✚ **Minimal medium**

- The media which lacks certain growth factors is known as Minimal Medium.
- Minimal Medium is mainly used for genetic experiments.

✚ **Ashby Medium**

- The Medium which lacks any nitrogenous compounds is known as the Ashby medium.
- Nitrogen-fixing bacteria are cultivated by using this type of medium.

✚ **Anaerobic Medium**

- Anaerobic bacteria require specialized media for extension because they demand low oxygen content, diminished oxidation-reduction potential, and more nutrients.
- This type of media ought to be enriched by nutrients such as hemin and vitamin k.

- Such media may also have to be overcome by physical or chemical means.
- Boiling the medium helps to discharge any suspended oxygen.
- The addition of 1% glucose, 0.1% thioglycollate, 0.1% ascorbic acid, 0.05% cysteine, or red hot iron fillings can provide a media reduction. Before applying the media needs to be sterilized in a water bath to dismiss any suspended oxygen and then sealed using liquid paraffin.
- Robertson Cooked Meal (RCM) medium that is generally applied to develop *Clostridium sp.* Carries a 2,5 cm column of bullock heart content and 15 ml of nutrient broth.

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