Bacterial culture media

A culture medium or growth medium is a liquid or gel designed to support the growth of microorganisms by providing nutrient requirements. There are different types of media suitable for growing different types of cells. Here, we will discuss microbiological cultures used for growing microbes, such as bacteria.

The nutrient requirement for bacteria:

1. **Carbon:** forms the backbone for the synthesis of small molecules in the cell and energy source.
2. **Nitrogen:** found in nitrogen-containing compounds, such as amino acids and nucleic acids.
3. **Phosphorus:** found in nucleic acids, ATP, and cytoplasmic membrane (phospholipids).
4. **Sulfur:** found in amino acids (cysteine and methionine) and some vitamins (thiamin).
5. **Other Elements:** Potassium, magnesium, and calcium are often required as enzyme cofactors.
6. **Trace Elements:** Zinc and Copper are found in enzymes that have metals as part of their structure.
7. **Oxygen:** Organisms that use (O_2) produce more energy from nutrients than anaerobes.

Classification of culture media

Bacterial culture media can be classified in at least three ways:

A. Classification based on physical state (consistency)

1. **Liquid media (Broth media):** is a medium lacking a solidifying agent. In a liquid medium, bacteria grow uniformly producing turbidity. These are available for use in test tubes. E.g. Nutrient broth. Broth medium is used for the propagation of a large number of organisms and various tests. Liquid media has some drawbacks. Properties of bacteria are not visible and the presence of more than one type of bacteria cannot be detected.
2. **Solid Media**: Any liquid medium can be rendered solid by the addition of certain solidifying agents. The most commonly used solidifying agent is agar. These are available for use in 3 shapes (plate, slant, and deep). E.g. Nutrient agar. Solid media is used for the isolation of bacteria as pure culture from mixed culture and Bacteria may be identified by studying the colony characteristics.

   Agar is a polysaccharide extract obtained from seaweed. It is an ideal solidifying agent (1.5-2%) as it is: (a) Bacteriologically inert, i.e. no influence on bacterial growth, (b) It remains solid at 37°C (melts at 98ºC and set at 42ºC, and (c) It is transparent. Before using agar for solidifying the medium, gelatin was used as a solidifying agent but it had some inherent problems. It existed as a liquid at normal incubating temperatures (35-37º C), it was digested by certain bacteria.
3. **Semi-solid agar:** Reducing the amount of agar to 0.2-0.5% renders a medium semi-solid. Such media are fairly soft and are useful in demonstrating bacterial motility and separating motile from non-motile strains.

Note: Besides agar, egg yolk and serum too can be used to solidify culture media. While serum and egg yolk are normally liquid, they can be rendered solid by coagulation using heat.

B. Classification based on the chemical composition

1. **Simple media** such as nutrient agar, peptone water can support most non-fastidious bacteria.

Note: Those bacteria that can grow with minimal requirements are said to be non-fastidious and those that require extra nutrients are said to be fastidious.

2. **Complex or non-synthetic media:** contain at least one ingredient that is not chemically definable such as blood agar has ingredients whose exact components are difficult to estimate.
3. **Synthetic Media:** These are chemically defined media prepared from pure chemical substances. It is used in research work.

C. **Classification based on functional use or application**

1. **Basal media** are simple media that supports most non-fastidious bacteria, they are used for the primary isolation of microorganisms. Examples: nutrient broth, and nutrient agar.
2. **Enriched media** the addition of extra nutrients in the form of blood, serum, egg yolk, etc. to the basal medium makes them enriched media. They are used to grow fastidious bacteria. E.g. Blood agar. Blood agar: is one of the most commonly used media. It may by adding (5-10) % sterile blood to any basic agar media (used to detect the hemolytic activity of bacteria, Chocolate agar (when blood agar is heated to 80 ºC for 10 minutes, the media change to chocolate brown color.
3. **Selective Media:** these media favor the growth of a particular bacterium by inhibiting the growth of undesired bacteria and allowing the growth of desirable bacteria. Examples: MacConkey agar, Tellurite media. Antibiotics may be added to a medium for inhibition.
4. **Indicator (Differential) Media:** an indicator is included in the medium. A particular organism causes a change in the indicator, e.g. blood, neutral red, tellurite. Examples: Blood agar and MacConkey agar .

MacConkey agar is:

1. Selective as bile salt allow the growth of Enterobacteriaceae but inhibits the growth of many other bacteria.
2. Indicators as the colonies that ferment lactose take a pink color due to production of acid. Acid turns the indicator neutral red to pink. These bacteria are called 'lactose fermenters', e.g. *E. coli*. Colorless colony indicates that lactose is not fermented, i.e. the bacterium is a non-lactose fermenter, e.g. *Salmonella.*

5. **Transport Media:** these media are used when a specimen cannot be cultured soon after collection. Examples: Stuart medium, Amies medium.
6. **Storage Media:** media used for storing the bacteria for a long period. Examples: Egg saline medium, chalk cooked meat broth.
7. **Assay Media:** these media are used for the assay of vitamins, amino acids, and antibiotics. E.g. antibiotic assay media are used for determining antibiotic potency.
8. **Reducing Media**: contains a substance (thioglycolic acid or cysteine) that absorbs O_2 or slows the penetration of O_2 in a medium. This type of medium is used for anaerobic bacteria.

Preparation of Agar Plate:

Most agars are present in powder form. They dissolved in distilled water as per their instructions as follow:

1. In a conical flask, Measure out a quantity of dry powdered nutrient media, add water, and check the pH (7).
2. Sealed the top mouth of the flask with a loose layer of aluminum foil.
3. Sterilize the prepared medium by autoclave. The autoclave exposes the media to high temperatures (121°C) and pressure (15 pounds per square inch (psi)) for 15 minutes.
4. The sterile media is then allowed to cool to (45 ^{o}C) pouring at this temperature to prevent condensation from forming on the lid.

Before plates are poured, every care is taken not to contaminate:

1. The bench was wiped with ethanol.
2. A Bunsen burner is set up with a gentle blue flame.
3. The number of plates is placed on the bench with their lids.
4. The aluminum foil, cotton are removed with a little finger.
5. The mouth of the flask is flamed to kill bacteria.
6. The lids of plates are lifted just enough to be poured, and are quickly half-filled with media.