

## Culture Media

**Microbial Growth:** Microbial growth involves an increase in the number of cells rather than in the size of individual cells. Growth of most microorganisms occurs by binary fission.

### Requirements for Growth:

❖ **Physical Requirements:** includes Temperature, pH and Osmotic Pressure.

❖ **Chemical Requirements:**

1. **Carbon:** Makes up 50% of dry weight of cell. Structural backbone of all organic compounds.

2. **Nitrogen, Sulfur, and Phosphorus:** **A. Nitrogen:** Makes up 14% of dry cell weight. Used to form amino acids, DNA, and RNA. **B. Sulfur:** Used to form proteins and some vitamins (thiamin and biotin). **C. Phosphorus:** Used to form DNA, RNA, ATP, and phospholipids.

3. **Other Elements:** Potassium, magnesium, and calcium are often required as enzyme cofactors.

4. **Trace Elements:** many are used as enzyme cofactors. Iron, Copper, Molybdenum, Zinc: found mainly in inorganic phosphate salts and buffers.

5. **Oxygen:** Organisms that use molecular oxygen (O<sub>2</sub>), produce more energy from nutrients than anaerobes.

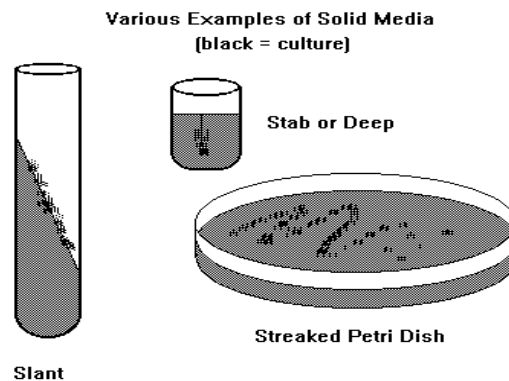
❖ **Culture Media:** A growth **medium** or **culture medium** is a liquid or gel designed to support the growth of microorganisms because it contains all the elements that most bacteria need for growth

**Types of culture media:** media can be classified on three primary levels:

### A. Physical state of media:

1. **Liquid medium (Broth medium):** is a medium lacking a solidifying matrix e.g; **Nutrient broth.**
2. **Solid Media:** Nutrient material that contains a solidifying agent. The most common solidifier is **agar**. Found in 3 shapes (**plate, slant and deep**).

**Unique Properties of Agar:** **a.** Melts 95<sup>0</sup> C. **b.** Once melted, does not solidify until it reaches 40°C. **c.** Cannot be degraded by most bacteria. **d.** Polysaccharide made by red algae.



**3. Semi- solid media:** they are semi-solid in room temperature, contain small amount of agar (0.3-0.5%). They used in the determination of bacterial motility.

### B. Chemical content of Media:

**i- Non-synthetic [chemically undefined] Natural medium** Chemically undefined ingredient may be broth or solid. Usually extracted from animals, plants or yeasts. e.g.: blood, serum.

**ii- Chemically defined [synthetic] medium:** A chemically defined medium is one prepared from purified ingredients and therefore whose exact composition is known as **MacConkey's Agar**.

**iii-Semi-synthetic medium:** some contents are chemically defined while others are undefined. e.g.: **Blood agar** or **Potato dextrose agar**.

### C. Functionally media can be:

**1. General media:** support the growth of all types of bacteria (pathogenic and non-pathogenic). E.g.: **Nutrient agar**.

**2. Selective Media** Inhibits the growth of some bacteria while allows for the growth of others. **Examples:**

**MacConkey Agar:** Most commonly used for Enterobacteriaceae. It contains agar, peptone, sodium chloride, bile salt, lactose and neutral red. It is a selective and indicator medium:

**(1) Selective** as bile salt does not inhibit the growth of Enterobacteriaceae but inhibits growth of many other bacteria.

(2) **Indicator medium** as the colonies of bacteria 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 fermenter', e.g. *Escherichia coli*.

Colorless colony indicates that lactose is not fermented, i.e. the bacterium is non-lactose fermenter, e.g. *Salmonella*, *Shigella*, *Vibrio*.

**EMB (Eosin-methylene blue) Agar:** A selective and differential medium for enteric Gram-negative rods. Lactose-fermenting colonies are colored and nonlactose-fermenting colonies are nonpigmented. Selects against gram positive bacteria.

**SS (Salmonella-Shigella) Agar:** It is a selective medium used to isolate *Salmonella* and *Shigella* species. SS Agar with additional bile salt is used if *Yersinia enterocolitica* is suspected.

**Mannitol-Salt Agar (MSA):** contains high levels of salts which inhibits growth of Gram negative bacteria and allows growth of Staphylococci.

**Saboraud's Dextrose Agar:** pH of 5.6 discourages bacterial growth. Used to isolate fungi.

**3. Differential Media:** contains substances that allow certain groups of bacteria to appear differently when growing on it. This allows us to determine if a certain bacterium is growing on the media by observing the appearance of the colonies. **E.g. Blood Agar:** Used to distinguish bacteria that destroy red blood cells (**hemolysis**).

**Blood Agar:** Most commonly used medium. 5-10% defibrinated sheep or horse blood is added to melted agar at 45-50°C. Blood acts as an enrichment material and also as an indicator. Certain bacteria when grown in blood agar produce hemolysis around their colonies. Certain bacteria produce no hemolysis. Types of changes: (A) beta (β) hemolysis. The colony is surrounded by a clear zone of complete hemolysis, e.g. *Streptococcus pyogenes* is beta hemolytic streptococci, (B) Alpha (α) hemolysis. The colony is surrounded by a zone of greenish discoloration due to formation of biliverdin, e.g. *Viridans streptococci*, (C) Gamma (γ) hemolysis, or, No hemolysis. There is no change in the medium surrounding the colony.

**4. Enriched media:** additional growth factors are added to the base medium. Used for growth of fastidious M.O. **e.g.: Blood agar and Chocolate agar.**

**Chocolate Agar or Heated Blood agar:** Prepared by heating blood agar. It is used for culture of pneumococcus, gonococcus, meningococcus and Haemophilus. Heating the blood inactivates inhibitor of growths.

**5. Enrichment Culture:** Used to favor the growth of a microbe that may be found in very small numbers. Used mainly for fecal and soil samples. E.g. Tetrathionate broth or Selenite broth.

**Tetrathionate Broth:** This medium is used for isolating *Salmonella* from stool. It acts as a selective medium. It inhibits normal intestinal bacteria and permits multiplication of *Salmonella*.

**6. Reducing medium:** is employed for growing obligate anaerobes. particularly contains chemicals (reducing agents) that deplete molecular oxygen e.g. **Thioglycolate broth.**

**Thioglycolate broth:** to test the oxygen requirements of microorganisms. Because thioglycolate tubes have an oxygen gradient, the location of growth in a tube indicates the oxygen requirement of an organism.

**7. Transport Media:** These media are used when specimen cannot be cultured soon after collection. Examples: Cary-Blair medium, Amies medium, Stuart medium.

**8. Storage Media:** Media used for storing the bacteria for a long period of time. Examples: Egg saline medium, chalk cooked meat broth.

### Preparation of culture media

- Measure out a quantity of dry powdered nutrient media, add water, check the pH (7) and boil.
- Sterilize the prepared medium by autoclave. The autoclave exposes the media to high temperature (121°C) and pressure (1.5 bar) for 20 minutes.
- Once the media is autoclaved, it is sterile (*all microorganism forms killed*).

