MICROBIOLOGY

Nutritional Requirements, Raw Materials used for culture media and Physical parameters for growth. Growth curve, isolation and preservation methods for pure cultures

PHARMACY INDIA

UNIT-1
PART-4

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B. PHARMA 5TH SEM ONE SHOT NOTES

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CLASSIFICATION OF CULTURE MEDIA
PHYSICAL OR ENVIRONMENTAL FACTORS AFFECTING BACTERIAL GROWTH
BACTERIAL GROWTH CURVE

CLASSIFICATION OF CULTURE MEDIA

A. CLASSIFICATION BASED ON NATURE OF ONSTITUENTS/INGREDIENTS

1. Those media that require **living cells or tissues** which are **parasitized** by the microorganisms to be cultured. A perfect example is cultivation of **Rickettsia** and **viruses** which are **obligate intracellular parasites** that require living host cells within which only they can multiply.
2. Those media that **doesn't** require **living cells or tissues.** These media may be divided into:
   - **Synthetic media or Defined Media -** These are the media in which only pure chemicals in definite concentrations are used. Due to their known chemical compositions, these media are useful for nutritional and metabolic studies.
   - **Non-synthetic media or Complex Media -** These are the media in which exact chemical composition of each of the constituents is not known with certainty.

B. CLASSIFICATION BASED ON CONSISTENCY (PHYSICAL SATE) OF MEDIUM

- **Liquid media -** These media are used in liquid form e.g. Nutrient Broth, Brucella Broth Nitrate Broth etc.
- **Semisolid media -** These media contain 0.5% or less of agar imparting custard consistency to the medium. e.g. Cysteine Trypticase Agar medium.
- **Liquefiable solid media -** These media are prepared by adding suitable amount of gelatin or agar to the liquid medium to remain solid when cool but become liquid when warm or vice-versa. For this reason, these media are also called "solid reversible to liquid media", e.g., Salmonella-Shigella Agar, Nutrient Gelatin Medium, Bile-Esculin Agar Medium etc.
- **Solid media -** These media always remain solid e.g., Nutrient Agar Medium, Bile Salt Agar Medium etc.

C. CLASSIFICATION BASED ON APPLICATION OR FUNCTION

- **Cultivation Media:** These are the media which are used for the general cultivation of bacteria e.g., Nutrient broth or nutrient agar is the most common cultivation medium used in microbiology laboratory.
- **Storage Media:** In such media, bacteria are stored as stock cultures for longer periods of time and hence serve as a source of viable cultures. Bacterial cultures are preserved and stored in such media by lyophilization(freeze-dry method).
- **Enrichment Media:** These are the media in which nutritional environment (may contain blood, serum, hemoglobin or special growth factors like vitamins and amino acids) is adjusted in such a way that the growth of certain bacterial types is enhanced selectively within a mixed population i.e., the media selectively favors the growth of one group of organisms over another. E.g., GN Broth, Salenite F, Loefller's serum slope etc.

D. DIFFERENTIAL OR INDICATOR MEDIA:

- These are the media which differentiate two organisms by their characteristic reactions towards the medium like hemolysis or biochemical changes.
- **For example -** Blood agar medium is an ideal differential medium which differentiates hemolytic species form non-hemolytic species e.g., ß-hemolytic (complete hemolysis in which clear/transparent zones are formed around colonies).

E. SELECTIVE AND SELECTIVE DIFFERENTIAL MEDIA:

- **Selective Media -** The media allows the growth of selective groups of organisms while inhibiting others by various inhibitors like antibiotics, dyes, bile salts etc. Important examples are MacConkey agar for isolation of E.coli, Deoxycholate citrate agar for Salmonella and Shigella, Lowenstein-Jensen medium for M. tuberculosis etc.
- **Selective Differential Media -** Some selective media play a dual role of selective growth of organisms and also differentiation of various groups of organisms on the basis of their biochemical reactions in a particular media. Such media are called selective differential media e.g., Blood Agar medium.

F. ASSAY MEDIA –

- These media are also called media for special purpose as these media (with known composition) have profound influence on the bacterial cells with respect to formation of enzymes, toxins, antibiotics and other bioactive products.
- E.g., Pyridoxine Deficient Growth medium for Streptococcus faecalis yields cells containing large amounts of tyrosine decarboxylase apoenzyme. Such media are used by technologists to test the effectiveness of antimicrobial drugs and to

assess the effect of disinfectants, antiseptics, cosmetics and preservatives on the growth of microorganisms.

G. **MAINTENANCE MEDIA -** These media are used to maintain physiological characteristics and hence the viability of bacterial cultures.

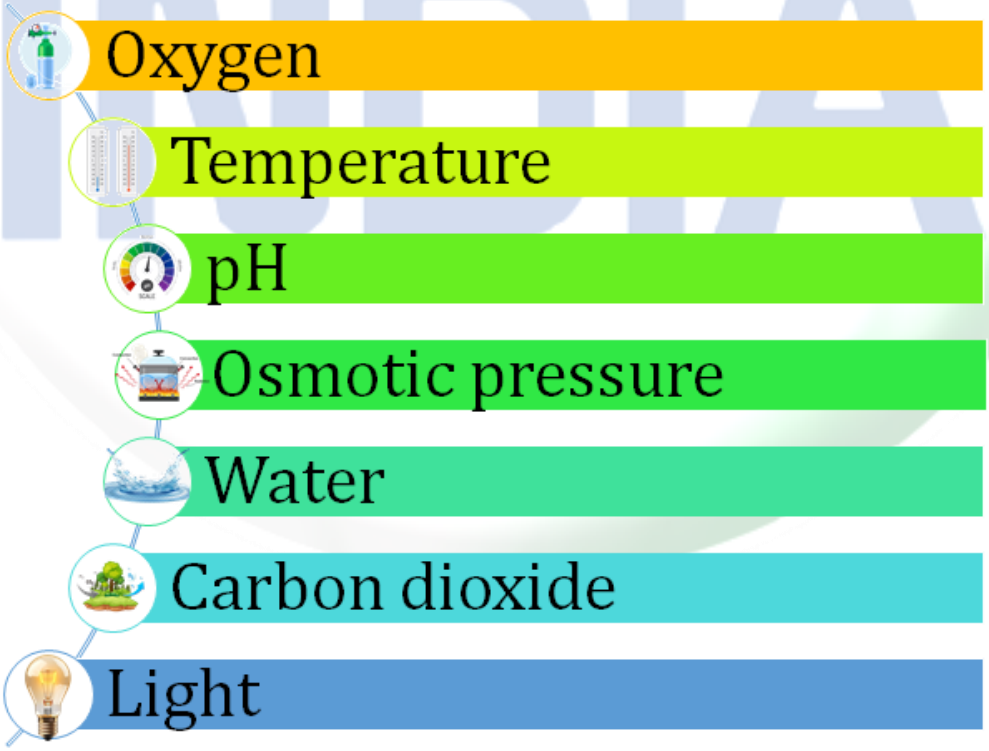
H. **TRANSPORT MEDIA –**

- These media **inhibit self-destruction** and **multiplication of microorganisms** and hence **maintaining the specimen** in a "status quo" condition. In other words these media are used to maintain and preserve specimens that are to be held for a longer period of time prior to clinical analysis.
- **Different types of specimen are transported in different transport media like;**
  A. Cary-Blair and Amies Transport Media
  B. Stuart Transport Medium
  C. Venkataraman-Ramakrishnan(V.R) medium
  D. Bile Peptone Transport medium

I. **ANAEROBIC MEDIA -** Such media are used for growth of anaerobes e.g. Robertson's cooked meat medium or Thioglycolate broth for isolation of Clostridium species.

J. **SUGAR MEDIA -** In such media sugar fermentation reactions are carried out which is important for identification of most of the organisms.

PHYSICAL OR ENVIRONMENTAL FACTORS AFFECTING BACTERIAL GROWTH



1. Oxygen

Oxygen is a universal component of cells and is always provided in large amounts by H_2O.

Bacteria differ in their requirement for molecular oxygen and are mainly divided into two groups;

a. **Aerobes -** They require oxygen for growth.

b. **Anaerobes -** They do not require oxygen for growth. They are further divided into following groups;

Obligate/stringent or strict aerobes: They only grow in presence of oxygen.

Obligate /stringent or strict anaerobes: They only grow in absence of oxygen.

Facultative/non-stringent aerobes: They are anaerobes that can grow even in presence of oxygen.

Facultative /non-stringent anaerobes: They are aerobes that can grow even in absence of oxygen.

2. Temperature

- It is the most important factor that determines the rate of growth, multiplication and death of all living organisms.
- A particular microorganism will exhibit a range of temperature over which it can grow, defined by three cardinal points in the same manner as pH.

Minimum temperature: It is the lowest temperature required for growth.

Maximum temperature: It is the highest temperature required for growth.

Optimum temperature: It is the temperature at which rapid growth in short period of time occurs.

3. Hydrogen ion Concentration (pH):

- The pH or hydrogen ion concentration[H*] of the growth medium of bacteria has a profound effect upon the multiplication of microorganisms.
- Each microbial species has a definite pH range and depending upon this optimum pH value, they can be classified as:
- These microorganisms have optimum pH ranges in between 1-6.5 and grow at an optimum pH well below neutrality.

- **Neutrophiles:** Most bacteria fall in this category and grow best in pH ranges between 6.5-7.5.
- **Alkalophiles:** These bacteria have an optimum pH ranges between 7.5-14.

4. **Osmotic Pressure**
   - Because of presence of cell wall, bacteria are able to with stand wide range of external osmotic pressure.
   - The only common solute in nature that occurs over a wide concentration range is salt (NaCl) 0.5% strength is added in most culture media for creating a suitable environment for bacterial growth.
   - On the basis of t osmotic concentrations of substrates upon which the micro-organisms grow, microorganisms are classified as;

Osmophobic - Those micro-organisms that die of dehydration if subjected to substrates of high osmotic concentrations.

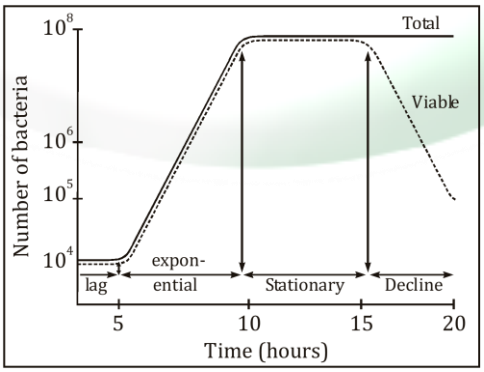
Osmophilic - Those microorganism that best grow on substrates of high osmotic concentrations.

Halophilic - Halophiles represent those microbes that preferably grow in high osmotic concentrations produced by dissolved salts.

Osmoduric - These are those microbes that grow normally on substrates of moderate osmotic concentrations but prove to be resistant to wide osmotic change in their substratum.

5. **Water -** Water is the solvent in which the molecules of life are dissolved, and the **availability of water is therefore a critical factor** that affects the bacteria growth as 80% of bacterial cell consists of water.
6. **Carbon dioxide -** Small amount of CO_2 is required by all bacteria which is usually made available endogenously (inside culture medium in **which bacteria is growing) as a product of cellular metabolism or by CO_2 present in atmosphere.**
7. **Light -** Bacteria (except phototrophic species) usually grow in darkness.

BACTERIAL GROWTH CURVE



S.NO.	PHASE	CHARACTERISTIC
1.	Lag phase	The period between inoculation and beginning of multiplication
2.	Log phase	• Reproduction and growth are at highest rate. • Number of bacteria doubles with each generation.
3.	Stationary	Reproduction and death rates equalize and hence bacterial population attains a plateau
4.	Death phase	Bacterial death due to lack of nutrition temperature and environment.