LAB2:

CULTURE MEDIA

Microorganisms, like all other living organisms, require basic nutrients for sustaining their life. All microorganisms have the same basic requirements but they are diverging in inorganic and organic compounds needs. By providing environmental and nutritional factors it is often possible to provide the appropriate conditions for their cultivation. On this basis we can define the **Culture media** as a fellow:

An artificial environment simulating natural conditions that necessary for bacteria to grow in laboratory.

Components of the Typical Culture Medium:

1- Energy source
2- Carbon source
3- Nitrogen source
4- Salts like phosphate, chlorides, sodium carbonates, potassium, magnesium, ferric, calcium and trace element like copper.
5- Source of different minerals e.g. iron, magnesium, sodium, potassium and traces of zinc and manganese.

Note: Some microorganisms may need a source of vitamins and amino acids which are important in building cellular components of microorganisms

Culture Media Importance:

1-Isolation and preservation of microorganisms.

2-Reproducing a microorganism and studying its characteristics.

3-Encouragement and induction of the microorganisms to produce materials that have industrial importance like antibiotics and some organic acids.

Classification of Culture Media:

culture media can be classified in at least three ways based on: consistency, nutritional component and its functional use.

A) Depending on its consistency:

1- Liquid media or broth: these are media that do not contain any solidifying agent like agar. Liquid media are suitable to grow bacteria when the numbers in the inoculum is suspected to be low, also they are usually used in the extraction of active compounds produced by microorganisms (e.g. toxins).

2- Solid media: these are media that contain (1.5 – 2 %) agar. Solid medium they are important for isolation of microorganisms in pure form especially when there is more than one species in one sample.

3) Semisolid media: it contains less than 1% (about 0.7 - 0.8) % of agar. Such media are soft and are useful in demonstrating bacterial motility and separating motile from non-motile strains, also their requirements for O_2 to know if these microorganisms are aerobic, anaerobic, micro aerobic, or facultative anaerobic.

Note: To obtain solid culture media, we use the following materials:

***Gelatin:**

It added to the medium in a percentage of (5-10) %. Its usage is limited because it liquefied at incubation temperature (37) °C and solidified at (25) °C. Furthermore, some kinds of bacteria can utilize it and decompose it because they have "gelatinase" enzyme.

***** Agar:**

A complex of carbohydrate material extracted from the red algae. It is considered a typical solidifying material because it doesn't have nutritional value for the bacteria. In addition, it liquefied at 100 °C and solidifies at 40- 45 °C.

B) Depending on its nature or components or contents:

1) **Natural media:** these are media that contain natural materials e.g. plant or animal tissues, milk, blood, fruit and vegetables juice and meat extract. The components of these media are accurately unknown.

2) **Artificial media:** these are divided into:

a) **Synthetic or defined media:** these are media which contain chemical substances that we know their composition and their concentration accurately. These media are used for

a wide variety of physiological studies.

b) **Semi synthetic or complex media:** these are synthetic media supplemented with natural components of unknown chemical composition like the addition of meat extract, yeast extract, peptone or serum.

Complex media are usually used for cultivation of bacterial pathogens and other fastidious bacteria.

3) **Living media:** these are media in which living cells are used as culture media like using chicken embryo and Hela cell for cultivation of viruses.

C) Depending on the purpose of uses:

1) **General purpose media:** these are media in which many are grown. They are used for many purposes e.g. nutrient broth.

2) **Selective media:** these are media that are used for the cultivation and isolation of certain species of microorganisms from a mixture of different species. These media are divided into two kinds:

A) Suppressive selective media: Selective media contain a component that helps to grow of a microorganism and suppresses the growth of other undesirable (un wanted) species.

There are several ways to suppress microorganisms like:

1- Addition of some suppressive materials to the medium like:

*The addition of certain dyes e.g. crystal violet, methylene blue, basic fuchsine which inhibit the growth of G +ve bacteria without affecting the G -ve growth.

*The addition of certain antibiotics e.g. cycloheximide which inhibits the growth of saprophytic fungi and allows the growth of fungi that have medical important like the Dermatophytes when it is added to Sabouraud agar.

2- By manipulation of certain growth conditions according to the growth conditions of the desirable species e.g. temperature, aeriation, and pH.

B) Enrichment selective media: these are media which are used for the selection of the desirable species of microorganisms by induction their

growth rather than other species which are grown in the same medium, this done by adding stimulatory materials which enrich the media like blood to nutrient agar medium to form blood agar medium. These media are used for the cultivation of fastidious bacteria.

3) **Differential media:** these are media which differentiate between two different groups of microorganisms and allow to diagnosis of microorganisms depending on its biological characters. Differential media contain certain material allows to detection of certain microorganisms depending on their metabolic activity.

***MacConkey agar:** it may concorded one of the first solid differential media that be formulated in the 20th century by Alfred Theodore MacConkey. MacConkey agar is a selective and differential media used for the isolation and differentiation of non-fastidious gram-negative rods, particularly members of the family Enterobacteriaceae, it differentiates between **lactose** and non-lactose fermented bacteria. The colonies of lactose fermented bacteria appear pink while the colonies of non-lactose fermented bacteria appear colorless. **Neutral red** is a pH indicator that turns red at a pH below 6.8 and is colorless at any pH greater than 6.8. Agar is the solidifying agent.

*Lactose fermented bacteria-------->pink colonies *E. coli*

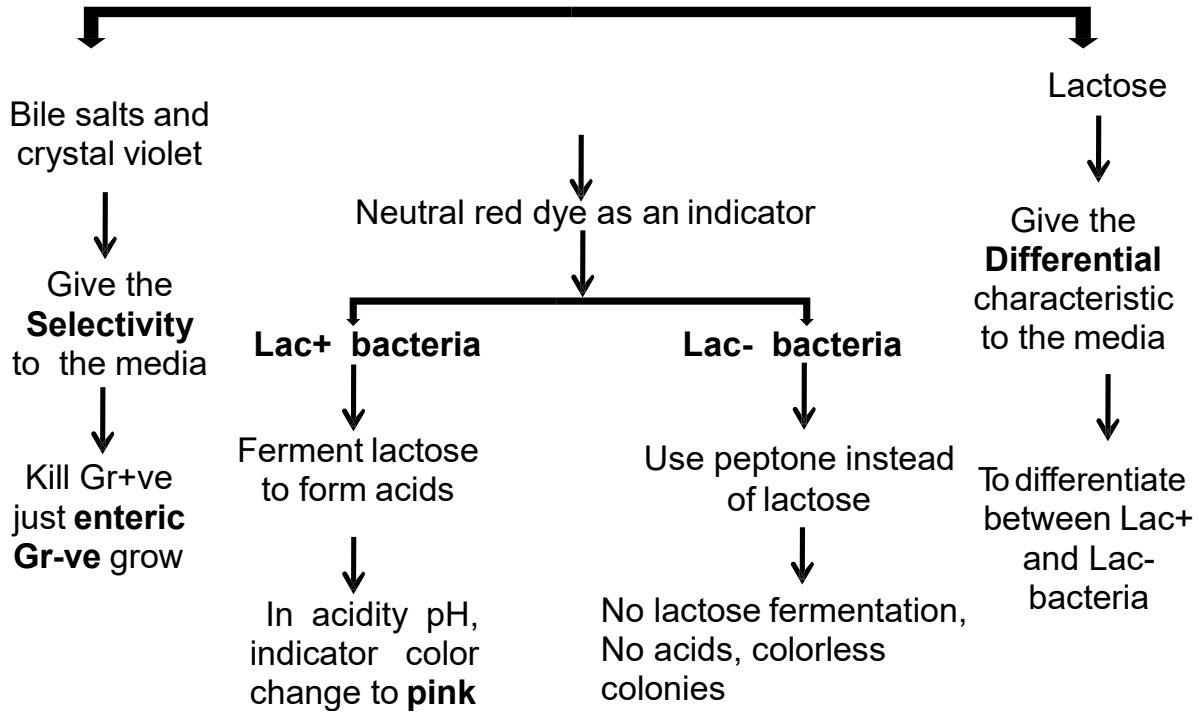
*Non-lactose fermented bacteria --------> pale or colorless colonies: *Neisseria*, *Proteus, Pseudomonas.*

<u>Principle of MacConkey Agar:</u> MacConkey agar is considered as a suppressive selective medium it permits the growth of Gr-ve enteric bacteria and inhibits the growth of Gr+ve non-enteric bacteria.

The selective action of this medium is attributed to the (crystal violet) which inhibits the Gr+ve bacteria, also the medium contains (bile salts) that inhibit non-enteric bacteria and both (crystal violet and bile salts) do not affect the growth of enteric Gr-ve bacteria because these bacteria is adaptable to live with the presence of bile salts in the intestine.

<u>Note: -</u> Many reagents or indicators are added to differential media to differentiate between different bacterial species that grow on same media. Usually, these reagents are dyes which detect the changes in the media acidity that result from microbial metabolic activity, the changing in acidity manifested by changes in the dye color. Thus, these reagents or dyes are called as pH-indicators.

MacConkey agar



4) **Maintenance media:** These media are used for maintenance and storage of microorganisms for long time by adding materials in a certain ratio. These materials maintain the persistence and viability of microorganisms for a longer time e.g. glycerol or tween-80 which leads to the slow growth rate of microorganisms (fast growth is followed by fast death). Maintenance media is preserved in the freezer. e.g. nutrient broth, brain-heart infusion broth.

5)Transport medium: Transport media are special media formulated to preserve a specimen and minimize bacterial overgrowth from the time of collection (human, soil, water, etc.) to the time it is received at the laboratory to be processed. Depending on the type of organisms suspected in the sample, transport media may vary. However, in general, transport media are classified based on physical state as semi solid and liquid and in basis of their utility as bacterial or viral transport media. Transport media contain only buffers and salt and doesn't contain any nutritional ingredients such as carbon, nitrogen, and organic growth factors to prevent microbial multiplication.

Example of transport medium:

Stuarts medium:

Commonly used for transporting specimens suspected of having gonococci. Also used for transporting Throat, vaginal, wound and skin swabs that may contain fastidious organisms.

Cary and Blair Medium:

semi-solid, white colored transport medium for faeces that may contain Salmonella, Shigella, Vibrio or Campylobacter.

5) **Assay media:** these media are used for performing assay like the medium that used for performing antibiotic sensitivity test which called Muller- Hinton agar.

6) **Stimulatory media:** these are media that stimulate the production of certain materials or structures inside the microorganism's cell like: toxins, pigments and endospores.

*Preparing the Culture Medium:

Note: Read the instructions found on the container of the culture medium carefully before preparing it.

1- Weigh the required amount from the powder of medium by using clean spatula, balance it and put it in a clean flask.
2- Add the required amount of distilled water to the flask.
3- Dissolve the medium powder in the distilled water by using hot plate with magnetic stirrer or by using the flame of benzene burner or without using heat.
4- Adjust the pH of the medium to the required value (+ 0.1 or + 0.2).
5- Distribute the medium in the tubes or flasks or any other containers according to necessity.
6- Sterilize the culture medium by autoclave
7- Cool the medium after sterilization and keep it in refrigerator at (4) °C until using it.

Note:

1-It not preferred to using cupper containers when prepare the medium, instead of use containers made of glass which are heating resistant.

2-It recommended to use distilled water rather than tap water for medium preparation.

3-It is preferred to adjust or fix medium pH before sterilization by autoclave.

Q) Why you adjust the pH of the medium before sterilization to the required value with the addition of +0.1 or +0.2?

The pH of the medium is adjusted before sterilization, so that it will be not contaminated when using pH-paper or when adding solutions used for fixing pH after sterilization. On the other hand, the reasons of increasing the value to the mentioned degree is related to the fact that pH or hydrogen ion concentration is affected by heat, it means that the concentration decreases when the temperature increases by heating medium in the autoclave (sterilization), therefore you must elevate (increase) the value before sterilization to reach the required value after sterilization.

Q) How do you adjust (fix) a medium pH?

1-Measure the medium pH either by pH paper or by pH- meter.

2-Adjustment medium pH to the desired degree as a follow:

3-add a few drops of 1 N NaOH to raise the pH or 1 N HCL to lower the pH. Be very careful to only add one drop at a time because it is very easy to over-adjust the pH

Note: You must cool the medium to (60) °C before the adjustment of the pH because the medium temperature affects the efficiency of the procedure especially when using the pH-meter.