By knowing environmental and nutritional factors that affect the growth of specific prokaryotes, it is often possible to provide the appropriate conditions for their cultivation.

**\*Culture media:** it is the environment where the microorganism lives and gets its own nutrients from nutritional materials forming that environment in order to grow & reproduce in laboratory

**\*Components of the Typical Culture Medium:**

2. Nitrogen source . 1. Carbon source. 3.Phosphate source. 4. Water source .

5. Source of different minerals e.g. iron, magnesium, sodium, potassium and traces of zinc and manganese.

**Note:** Some m.o. may need a source of vitamins and amino acids which are important in building cellular components of m.o.

**\*Culture Media Importance:**

1) Isolation and preservation of m.o

2) Reproducing a m.o and studying its characteristics.

3) Encouragement and induction of the m.o to produce materials of industrial importance like production of antibiotics and some organic acids.

**\*Division of Culture Media :**

A// Depending on its consistency:

1) Liquid media or broth: these are media that do not contain any percentage of agar.

They are usually used in the extraction of active compounds produced m.o e.g . toxins

2) Solid media: these are media that contain (1.5 - 2) %. agar. They are used for the isolation of m.o in the form of pure colonies and for isolation of two kinds (or more) of bacteria.

3) Semisolid media: these are media that contain less than 1% of agar, about(0.7 – 0.8) %.

This amount of agar is added to the liquid medium so it becomes gelatinous. These media are used for studying of the bacterial motility and their demands for O2 in order to know if these m.o are aerobic , anaerobic , micro aerobic , or facultative anaerobic .

**Note:** In order to obtain solid culture media , we use the following materials

1)) Gelatin:

1- It is added to the medium in a percentage of (5-10) %.

2- Its usage is limited because it liquefies at incubation temperature (37)ºC and solidifies at (25)ºC. Further, some kinds of bacteria can utilize it and dissociate it because they have "gelatinase" enzyme .

2)) Agar:

1- A complex carbohydrate material extracted from the red algae.

2- It is considered a typical solidifying material because it doesn't have nutritional value for the bacteria,

so it isn’t attached or utilized by the bacteria. In addition it liquefies at (100) ºC and solidifies at (40- 45) º.

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 , diluted blood , fruit and vegetables juice ( like tomato and potato) and meat extract . The components of these media are accurately unknown e.g. tissue culture which is prepared from chicken embryo and used for cultivation of viruses.

2) Artificial media: these are divided into:

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

1- The nutritional requirements of m.o .

2- The effect of each of the substances forming these media on m.o .

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.

These media are used when the studied bacteria are sensitive to synthetic media so they are cultured or grown on media supported with natural sources.

**Note:** Synthetic media are used for cultivation of fastidious bacteria while semi synthetic media are used for cultivation of non-fastidious bacteria.

3) Living media: these are media in which living cells are used as culture media like the use of chicken embryo for cultivation of viruses.

C//Depending on the purpose of uses:

1) General purpose media: these are media in which many m.o 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 m.o from a mixture of different species .

These media are divided into two kinds :

A) Depressive selective media: these are media which are used for the selection of the certain species of m.o by depressing the undesirable (un wanted ) species . There are several ways of depression like:

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

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

b- The addition of certain antibiotics e.g. cycloheximide which inhibits the growth of saprophytic fungi and allows the growth of medical fungi when it is added to sabouraud agar .

2- Using certain growth conditions and changing them according to the growth

conditions of the desirable species e.g. temperature , ventilation, and pH.

B) Enrichment selective media: these are media which are used for the selection of the

desirable species of m.o. by induction the growth of these species in a better way

than the other species which are grown in the same medium and that is done by

adding stimulatory materials which enrich the media like the addition of blood to the

nutrient agar medium in order to form blood agar medium. These media are used for

the cultivation of fastidious bacteria.

3) Differential or identification media: these are media which differentiate between two different groups of m.o. and allow the diagnosis of m.o. depending on its biological characters (it means that these media contain certain material allows the detection of certain m.o.depending on a metabolic actiondone by that m.o.) e.g.

MacConkey agar which differentiate between lactose fermented bacteria and non-lactose fermented. The colonies of lactose fermented bacteria appear pink while the colonies of non-lactose fermented bacteria appear colorless (it means that these colonies have a color which is similar to the culture media color).

\*Lactose fermented bacteria ---------< pink colonies: E . coli

\*Non-lactose fermented b. --------> pale or colorless colonies: Neisseria , Proteus

Pseudomonas

\*pH - indicator----------> neutral red

\*Sugar --------> lactose

**Note**: - MacConkey agar is considered a depressive selective medium

it permits the growth of G-ve enteric bacteria and inhibits the growth of G+ve non-enteric bacteria.

Why? This medium contains (crystal violet) which is a dye that inhibits G+ve bacteria,

and the medium is also contains (bile salts) which inhibit non-enteric bacteria and both of them (crystal violet and bile salts) do not affect the growth of enteric G-ve bacteria because this bacteria is adaptable to live with the presence of bile salts in the intestine .

**Note:-** Reagents or indicators are added to the differential media to differentiate between different types of m.o. which are grown on these media. Usually these reagents are dyes which investigate the change of the medium acidity as a result of a metabolic action done by the m.o. and this ehange in acidity is manifested by noticing the color change of the reagent ( the dye ) which is added to the medium and these reagents are called (PH-indicators).

MacConkey agar Differential m. ----> Neutral red G-ve √

Depressive selective m. Crystal violet

G+ve X

Bile salts

Enteric b. √ Non-enteric b. X

4) Maintenance media: these media are used formaintenance and storage of m.o. for

along period by adding materials in a certain ratio. These materials maintain the persistence and viability of m.o. for a longer time e.g. glycerol or tween-80 which leads to the slow growth of m.o. because fast growth is followed by fast death and that is not desirable. There is a special medium for the maintenance of each m.o.and that medium is maintained in the freezer e.g. nutrient broth, brain-heart infusion broth.

5) Transport medium: these media that are cultivated with the sample temporarily in order to transport it from its isolation source (human, soil, water, ….etc)

to the laboratory for maintaining and keeping its viability and other characteristics e.g. stuart medium which is used for transporting Gonnorhoea bacteria (Gonococci) and glycerol saline medium which is used for transporting stool samples.

6) Assay media: these media are used for performing a particular test (assay) like the medium that is used for performing antibiotic sensitivity test which is called Muller- Hinton age.

7) Stimulatory media: these are media that stimulate the production of certain materials or structures inside the m.o. cell like: toxins, pigments and endospores.

**\*Preparing the Culture Medium:**

**Note:** You must read the instructions found on the container of the culture medium before preparing it .

1. Weigh the required amount of the medium powder by using the balance and put it in a flask or other containers.
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) ºC until using it.

**Note:**

1- It is preferred not using containers that made of cupper when you prepare the medium, instead of these you can use containers made of glass which are heat resistant.

2- It is preferred using the distilled water for instead of the tap water for preparation of medium.

3- It is preferred to adjust or fix the pH of the medium before sterilizing it in 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) the medium pH ?

1. By using the pH - indicator paper.

2. By using the pH- meter.

\*Adjustment of the medium pH is done as follows:-

1. By adding a couple of drops of NaOH in the concentration of 1N .

2. By adding a couple of drops of HCl in the same concentration.

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