Lab: 4 *Media Preparation*

Culture Media

Every students of microbiology must master certain basic techniques, including:

1. Preparation and sterilization of a suitable medium that will support microbial growth.
2. Aseptic handling and transfer of microbial cells
3. Isolation of M.O and the establishment of pure cultures.
4. The establishment and preservation of a stock culture to serve as a source of inoculum for further studies.

Culture Medium:

Collection of certain basic nutrienal requirements that provide cultivation of bacteria and support their growth and multiplication, which include:

1. Carbon source that may also serve as an energy sources.
2. Water .
3. Nitrogen source.
4. Phosphate source.
5. Various mineral nutrients such as iron & Mg .

Pure Culture:

Culture consist of medium containing the growth of a single species of M.O which involve in most microbiological studies and industries.

Mixed Culture:

Culture consist of medium containing the growth of two or more species of M.O.

There are several ways to ensure the purity of a culture:

a) Colony should only contain a single morphological cell type when examine under microscope.

b) Isolated M.O from streak plate should yield only a single type of isolated colony which contain the same morphological type of cells.

- Pure culture preservation:

1) Cooling

2) Freezing

3) Lyophilization (Freeze drying).

Kinds of Culture Media:

- According to their consistency:

1. Broth or liquid media :

Is one in which the components are simply dissolved in water (without solidifying agent), for example: Nutrient broth (N.b), Brain-heart infusion broth, The benefit of the broth is to propagate large no. of M.O for various biochemical Test & fermentation studies.

2. Solid Media :

Is one in which the components in addition to(agar, (as a solidifying agent), dissolved in water. Many microbiological media contain (1.5 - 3 % agar), for example: Nutrient agar Solid media are used to obtain surface growth in order to observe colony appearance for:

1) Pure culture isolation

2) Storage of cultures as slants

3) Observe specific biochemical reactions .

3. Semi- Solid Media:

As the solid media but the proportion of agar is 0.3 – 0.5%. which are used for:

1) Fermentation studies

2) Determining bacterial motility

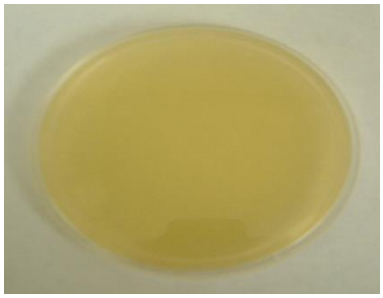
3)Promoting anaerobic growth

N.b = Beef extract + peptone (hydrolyzed protein) + NaCl Nutrient agar = same components of N.b + agar (1.5-3%).

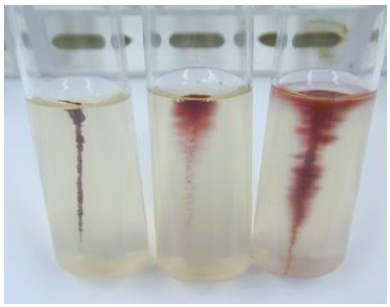
Agar : is a complex carbohydrate extracted from sea weed Gelidium an ideal solidifying agent for microbiological media because of:

1) It is melting properties(solid agar melts at 90-100 C, liquid agar, solidified at about(42 C).

2) Because it has no nutritive value for majority of bacteria.



Soid medium



Semi-soild medium

Liquid medium

2-According to their uses & content:

1. Natural : (non- synthetic media)

Media are consist of complex nutritionals that are rich in vitamins & nutrients such as: milk, blood. they used according to the test not by knowing the extract chemical composition.

2. Defined: (synthetic media):-

The exact chemical composition is knowing and highly purified, this medium consisting of single carbon source, such as glucose. A nitrogen source, such as ammonium salts and inorganic salts, such a phosphate. It is used for growing certain species of M.O.

3. Semi- synthetic media :

Composed of synthetic medium with natural substances, for example: Nutrient agar with blood = Blood agar.

4. Living media :

Consist of living tissue, used for growing viruses and cancer cells, for example: Tissue culture and Volunteers.

5. Enrichment media:

The basic medium can be enriched to support the growth of more fastidious types of bacteria by the addition of materials such as blood, plasma or yeast extract which provide a variety of complex nutritional factors. Eg: Blood agar, Chocolate agar .

Blood agar

Chocolate agar

6. Selective media :

Permit the isolation of specific groups of M.O. this medium incorporate chemical compounds that inhibit the growth of one type of M.O while permitting the growth of another type, the inhibitory substances may be salt

NaCl, acid, a toxic chemical (Crystal violet), an antibiotic (streptomycin)...etc., For example: MacConkey agar.

7. Differential medium :

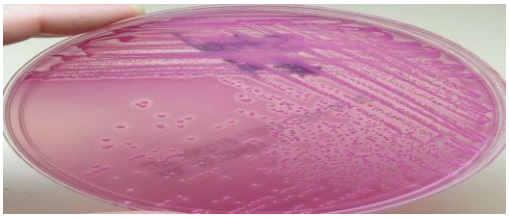
Permit the recognition of specific types of M.O. that grow on the medium. Differential medium incorporate substances that produce recognizable reactions and therapy permit the differentiation of particular types of M.O among others growing on the same media, for example: MacConkey agar.

8. Assay Media
9. Media for enumeration of bacteria
10. Media for characterization of bacteria

► **MacConkey's** (MAC)

media is both **selective & differential**.MacConkey

- **Selective** because it *only grows* ***Gram-negative****bacteria*. Inhibits the growth of **Gram-positive** bacteria.
- **Differential** because neutral red (pH-sensitive dye) and lactose (type of sugar) have been added to media.

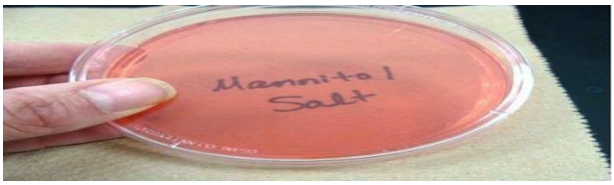


► **Mannitol Salt** (MSA)

Mannitol Salt media is both selective & differential.

1-Selective because it has a high NaCl (7.5%) concentration, and few types of bacteria can grow on this hypertonic medium

2. Differential because this medium contains a pH-sensitive dye to identify organisms that ferment mannitol. O



Preparation of Culture Media:

1) Weighing the medium ingredients and mixing them:

• To prepare 500 ml of N.b (wrote on its container 8 mg/L)

The suitable weight is:

8gm 1000ml(D.W)

X **500**ml(D.W)

X=8 * 500 = 4 gm/ml

1000

500 ml. D.W

Weight 4 gm of N.b and add it D.W, Dissolve by agitation (heat may be necessary). After complete dissolution, bring the volume to 500 ml by adding D.W

2) Adjusting pH: Check the pH of each flask using pH indicator or paper or pH meter.

just the pH to 7 if necessary by adding drop by drop, either 1 N NaOH or N HCI.

The N.A should be cooled to about 55-60 C before attempting to adjust its pH to avoid any change in the pH of medium, we must use buffer salts.

Adding the medium to the test tubes: • By pipetting dispense broth medium (or agar medium used as slant afterward) into test tubes, lable the tubes.

Note: media are sterilized by autoclaving at 1210C and 2 atmosphere for 15-20 minutes. With the autoclave, all bacteria, fungi, viruses, and spores are destroyed. Some media can't be sterilized by autoclaving because they contain eggs or carbohydrates .