Lab 3

CULTURE MEDIA

By
Assistant Lecturer
Anfal I. Alawadi

Culture media :are mediums that provide essential nutrients and minerals to support the growth of microorganisms in the laboratory.

Common ingredient for culture media

- **Bacteria like any living cell it need organic & inorganic material for their live. so in order to propagate these bacteria we should provide them with these material by :-**

1- peptone: source of protein.

2-Nitrogen source(beef extract)

3-Nacl : for isotonic environment

4- H2O

5-Agar : no nutritional benefits but used for solidifying purposes

6-PH : most pathogenic needs a PH 7.2-7.4

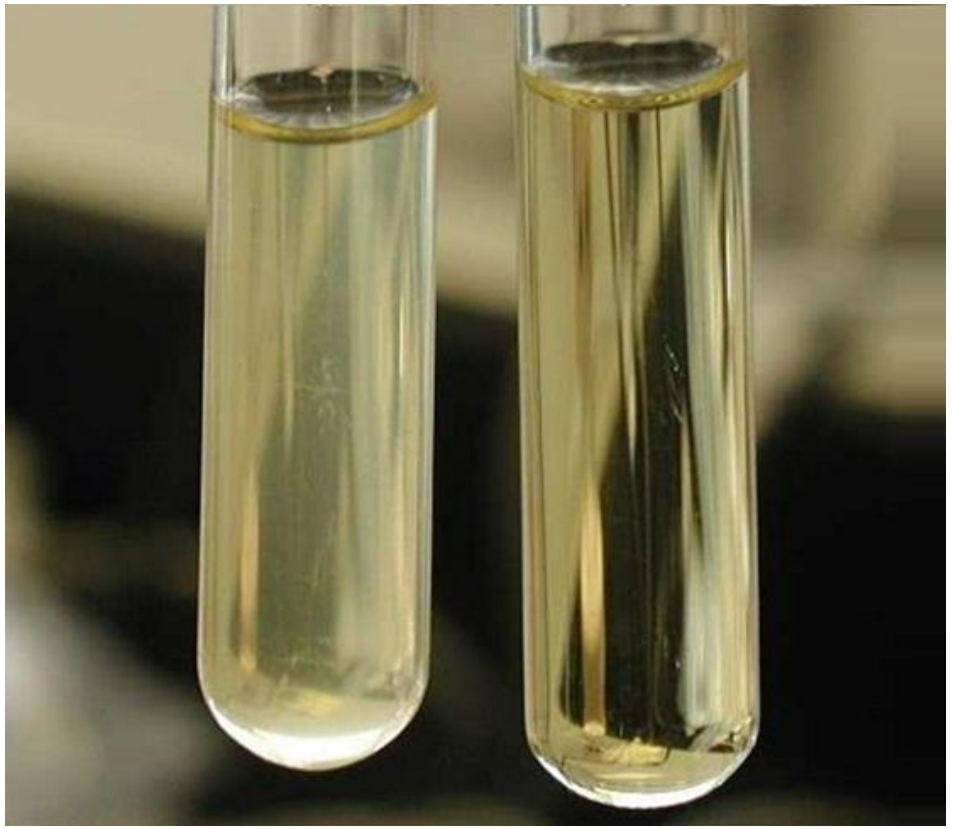
Classification

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

1) Classification based on consistency:

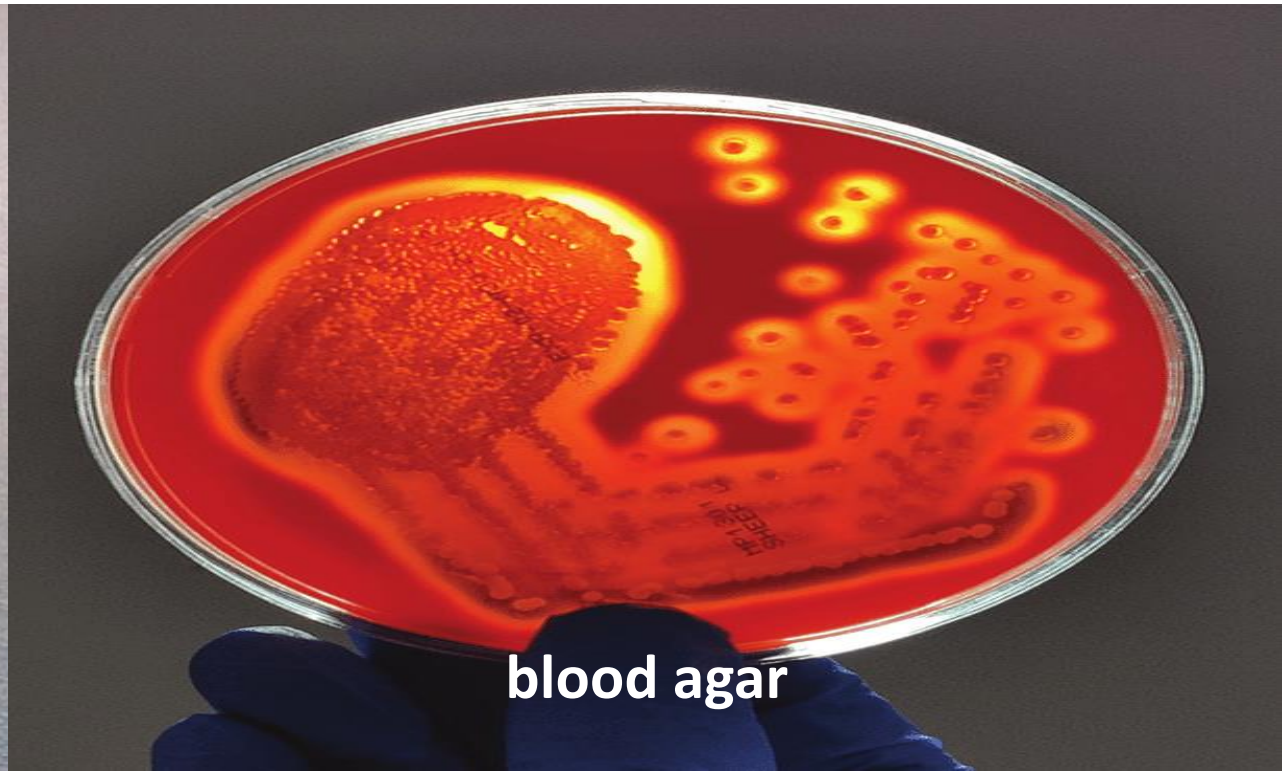
A-liquid, B- semi-solid , C-solid .

- **A) Liquid media: Liquid media are sometimes referred as “broths” (e.g nutrient broth). In liquid medium, bacteria grow uniformly producing general turbidity.**
- **use in test-tubes, bottles or flask**



B) Solid media: Any liquid medium with the addition of certain solidifying agents. Agar agar (simply called agar) is the most commonly used solidifying agent.

- **it is used at concentration of 1-3% to make a solid agar medium.**
- **use for identification of colony morphology e.x:(nutrient agar, Macconkeys agar , blood agar, if heated for 70c it will yields chocolate agar).**



C) Semi-solid agar: Reducing the amount of agar to 0.2-0.5% renders a medium semi-solid.

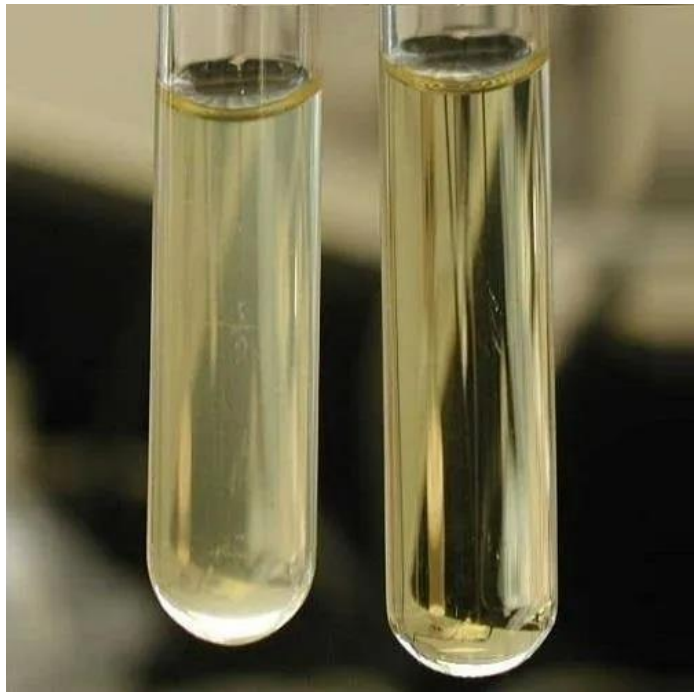
This media are fairly soft and are useful in demonstrating bacterial motility and separating motile from non-motile.

Example : Mannitol motility media

Mannitol motility media

2) Classification based on nutritional component:

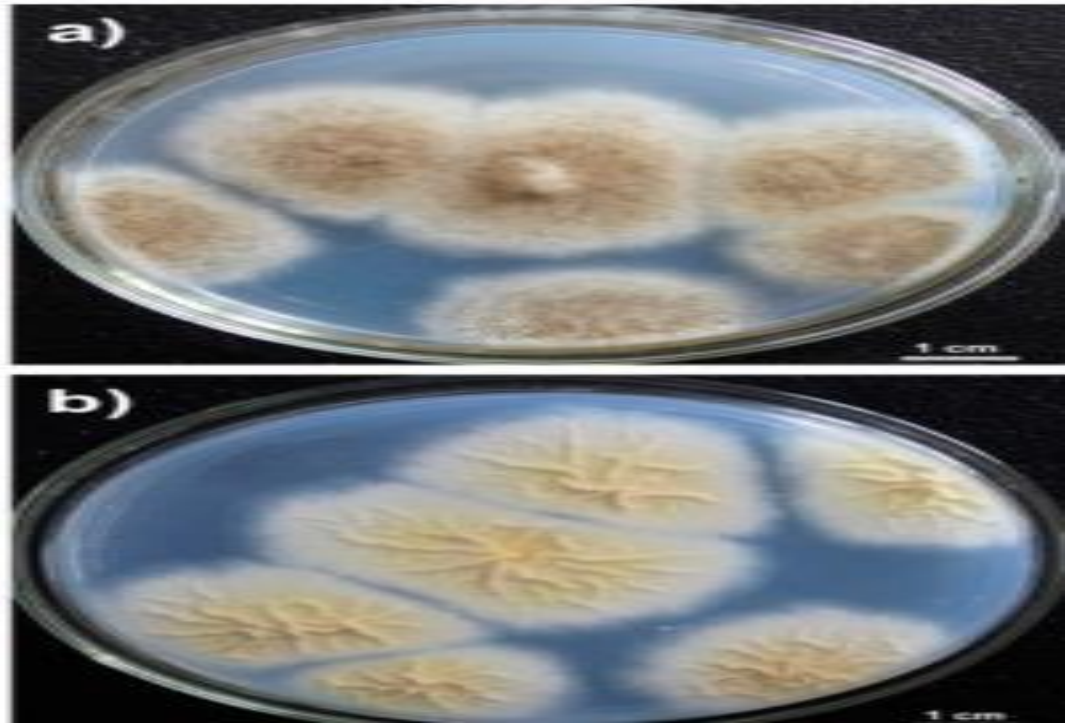
- Media can be classified as simple, complex and synthetic (or defined). A:Simple media such as peptone water, nutrient agar, nutrient broth.



nutrient broth

nutrient agar

- **B:Complex media** such as blood agar.
- **C:Synthetic or defined media** and is produced from pure chemical substances.such as Davis, Czapek Dox Medium & Mingioli medium are specially prepared media for research purposes where the composition of every component is well known.



Aspergillus tubingensis **growing on Czapek medium**

3) Classification based on functional use or application:

1)Basal media . this media contains only common components such as carbon and nitrogen sources that boost the growth of many microorganisms and used for cultivation of non fastidious M.O

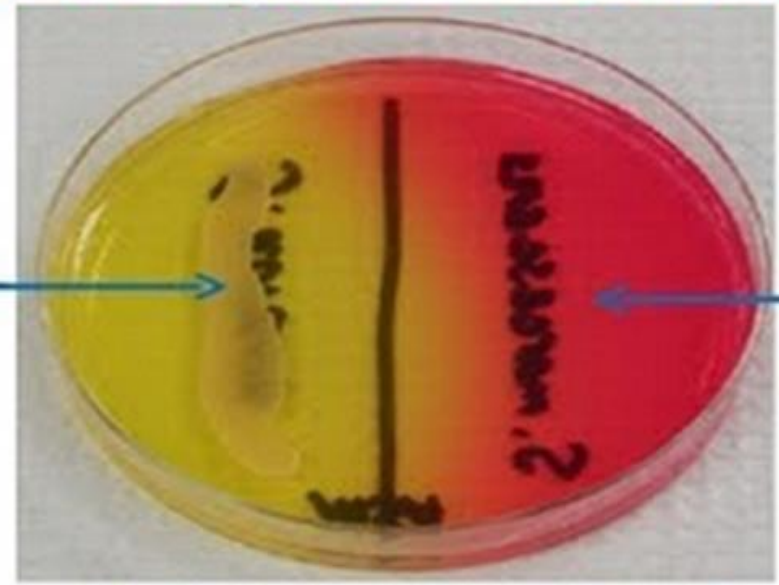
ex. Peptone water, nutrient broth and nutrient agar considered basal medium.

2) Enriched media: Addition of blood, serum, egg yolk etc, to basal medium makes them enriched media. Enriched media are used to grow (fastidious) bacteria.

ex. Blood agar, chocolate agar.

3)Selective media:-culture medium that allows the growth types of organisms, while inhibiting the growth of other organisms . these media contain substance (e.g. bile salt or other chemical and antibiotics) which inhibit the growth of one organism to allow the growth of another, e.g. mannitol salt agar contain a high concentration of sodium chloride NaCl 7% that inhibits the growth of most organism but permits staphylococci to grow.

Mannitol salt agar



Staphylococcus aureus grows

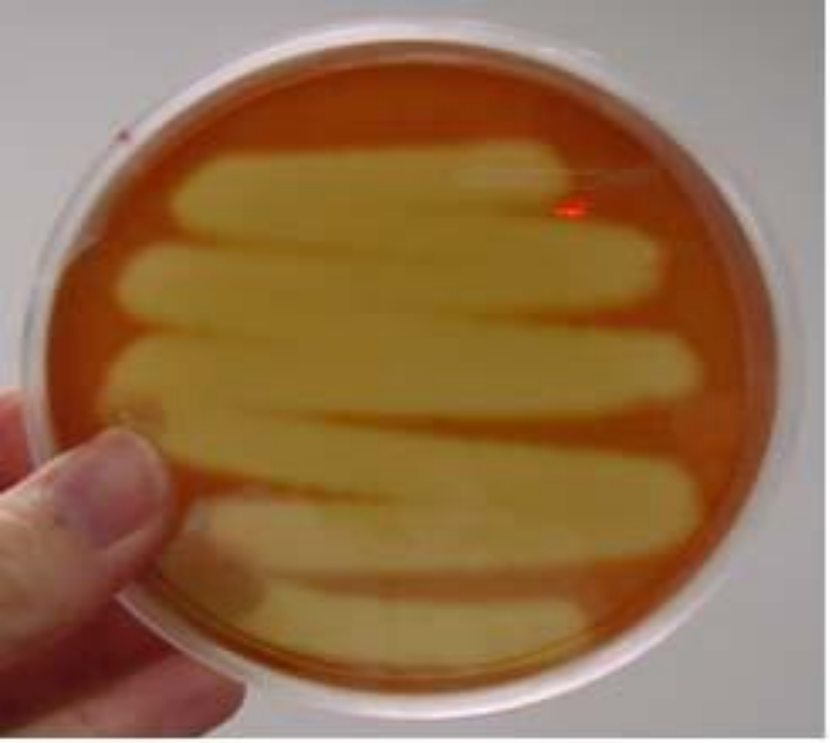
Serratia marcescens does not grow

Selective medium

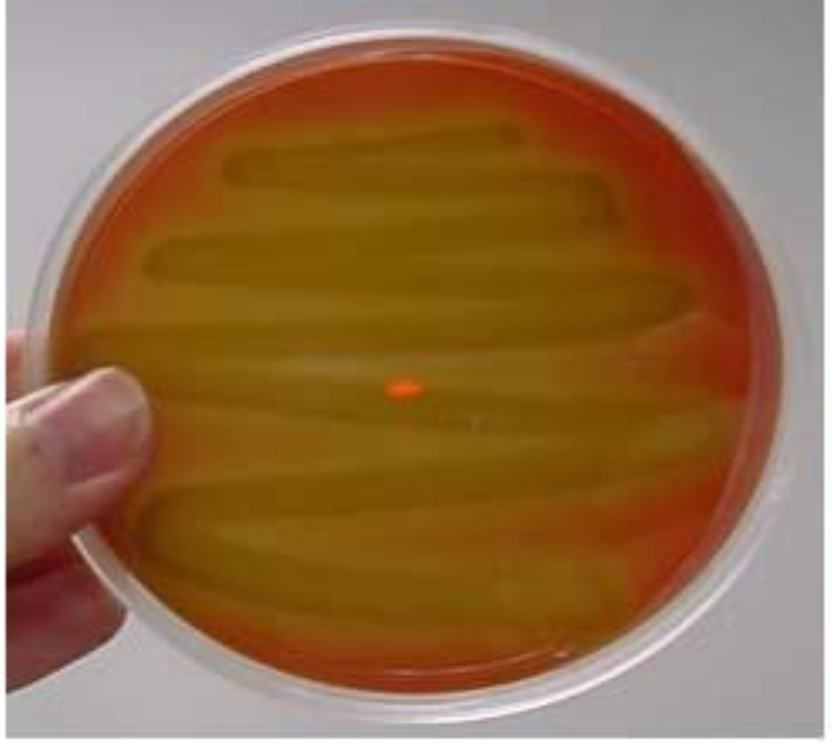
High salt (NaCl) concentration in medium favors organisms that tolerate high salt concentration, e.g. *Staphylococcus*.

4)Differential media:- **culture medium that includes ingredients, such as chemical indicators that produce observable differences between species of bacteria. EX, blood agar**

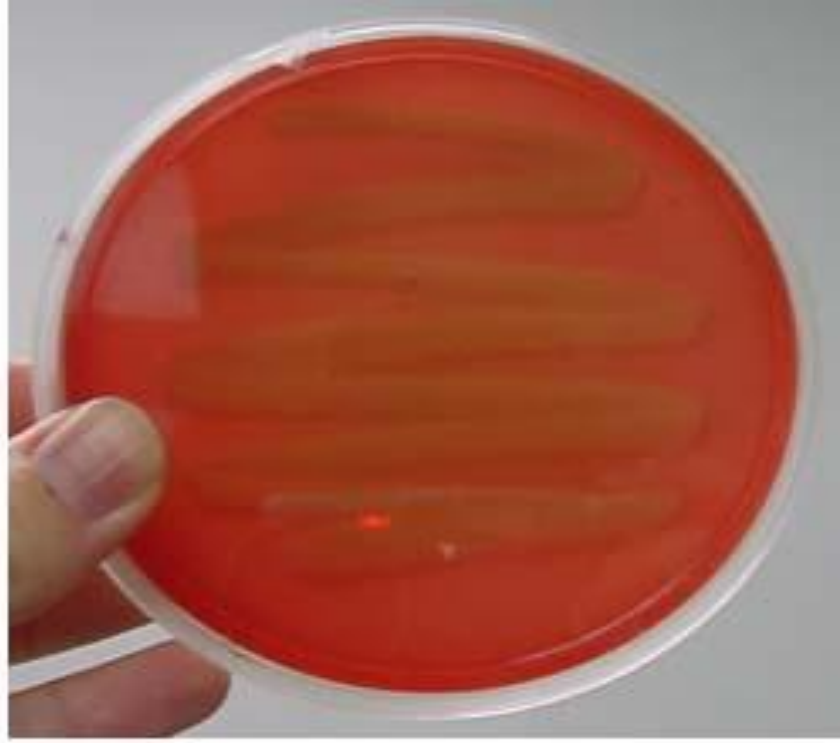
- **This media is differential because:**
- **Certain bacteria produce enzymes (hemolysis…) that act on the red cells to produce either:**
- **Beta hemolysis: Enzymes lyse the blood cells completely, producing a clear area around the colony.**
- **Alpha hemolysis: Incomplete hemolysis produces a greenish discoloration around the colony**
- **Gamma hemolysis: No effect on the red cells.**



Beta Hemolysis



Alpha Hemolysis



Gamma Hemolysis

BETA HAEMOLYSIS Streptococcus pyogens

ALPHA HAEMOLYSIS Streptococcus pneumonia

GAMMA HEMOLYSIS Streptococcus bovis

- **5) Transport media: certain microorganism is weak & die rapidly between the time taken of the specimens and examination so its needs a special media for transport**
- **Such media prevent drying (desiccation) of specimen.**
- **Ex , Stuarts media .**

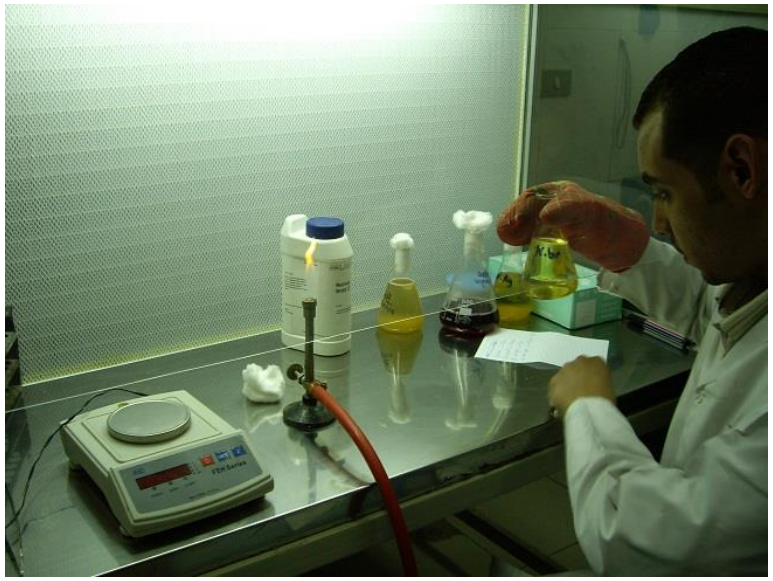
6).Anaerobic media:- This media is for anaerobic bacteria which require low oxygen levels. Examples are: **Thioglycollate broth** which is commonly used to grow Clostridium spp.

7) Sensitivity media :- a special media used to test antibiotic sensitivity for given m.o Muller Hinton media

How to prepare media?

- 1) Measure out a quantity of **dry powdered nutrient media**, add **water** and **check the pH(7)**.
- 2)They dispense the media into bottles(flask ,tube), cap it and **autoclave**. The autoclave exposes the media to high temperature (121°C) and pressure (15 psi) for 20 minutes.
- Once the media is autoclaved it is sterile.
- 3)Pouring in petri dish and leave for 5-10 minutes to solidify .
- 4)The plates are ready for inoculation and cultivation


OXOID
CM0337
MUELLER-HINTON
AGAR




200 mL
±5%
150
100
50

Aseptically pouring agar plates

Agar plates are stored upside down to prevent condensation.

Thank you