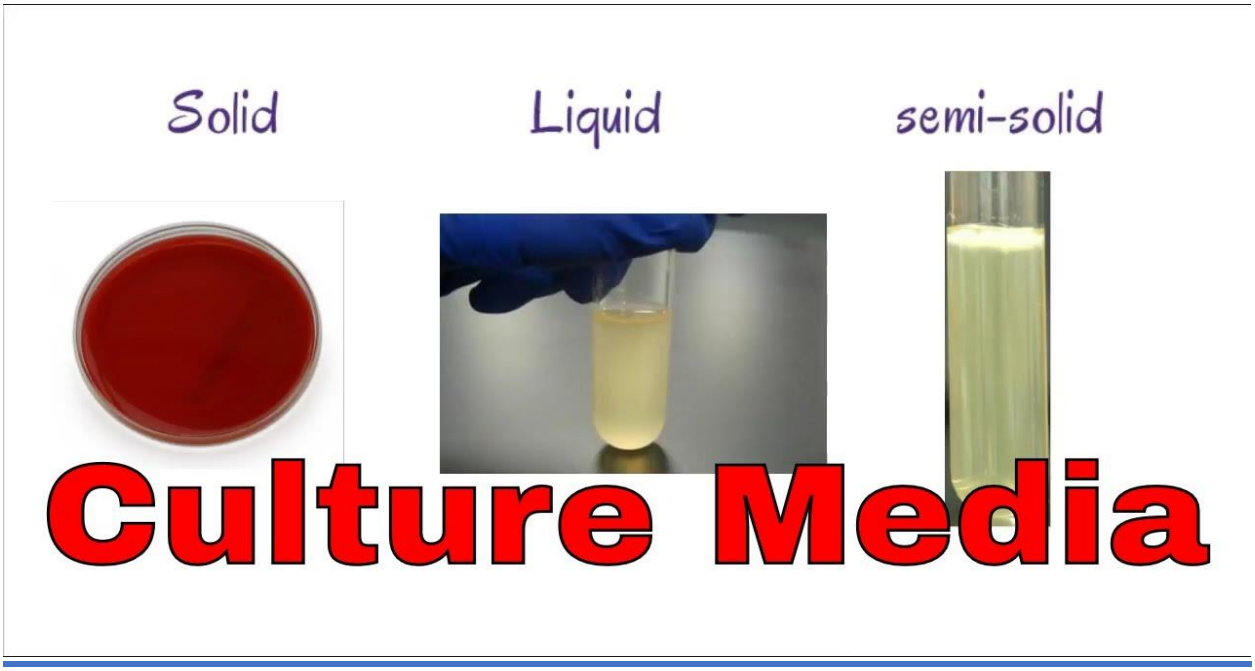
LAB-2 **Practical pathogenic bacteria**

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 is divided according to the purpose of its use

▶ 1) 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.

▶ B)Enrichment selective media:

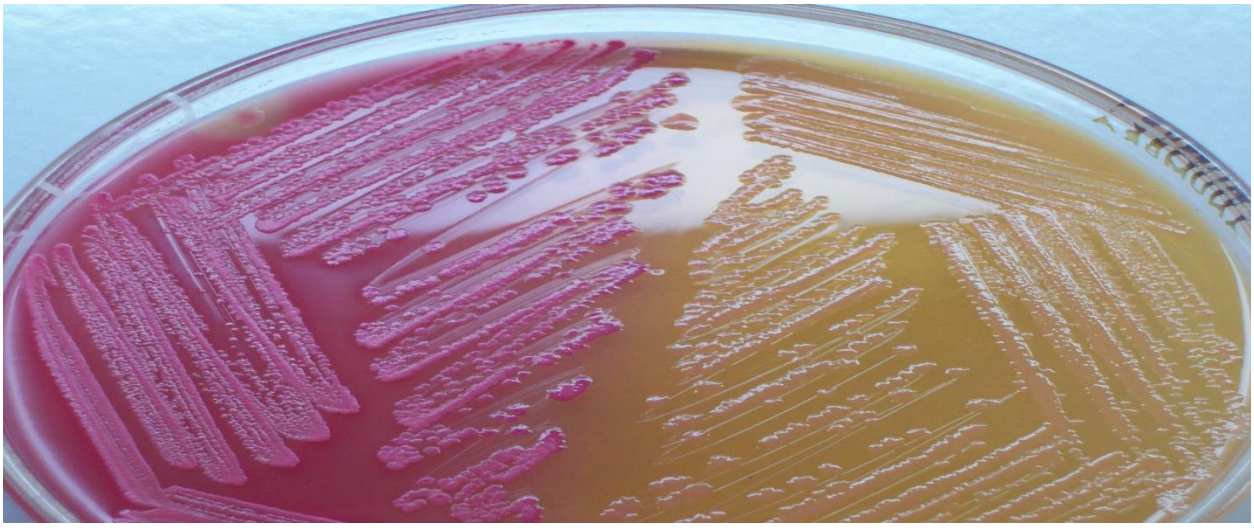
these are media which are used for the selection of the desirable species of microorganisms by induction their growth rather than other species which are grown in the same medium, this done by adding stimulatory materials which enrich the media like blood to nutrient agar medium to form blood agar medium.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

▶ 2)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.

- **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, and 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.**

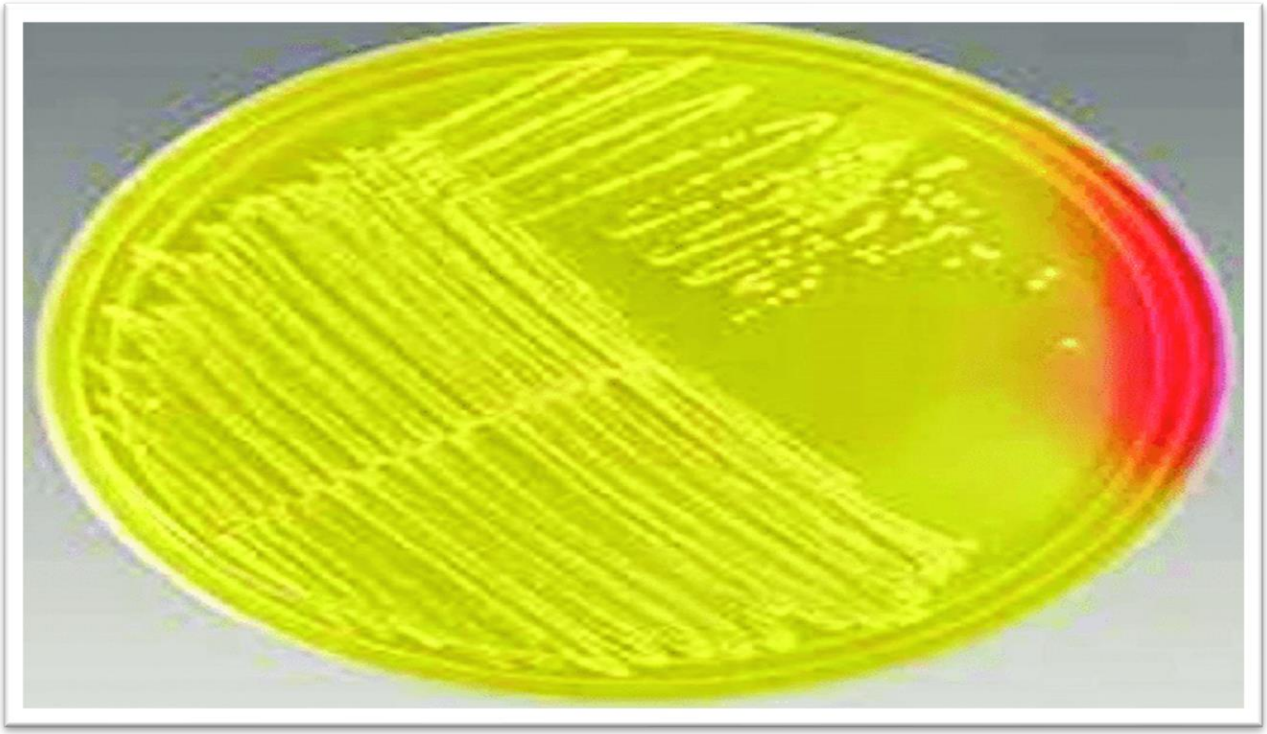
- **MacConkey agar (selective differential media for gram negative bacteria)**
- **Sugar : Lactose**
- **PH-indicator: Neutral red**
- **The inhibitor for gram positive bacteria : crystal violet**
- **The inhibitor for non-enteric bacteria : bile salt**
- **Lactose fermenter = pink colony**
- **Lactose non fermenter = pale colony**



- **Principle of MacConkey Agar: 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.**

- **Note: - 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.**

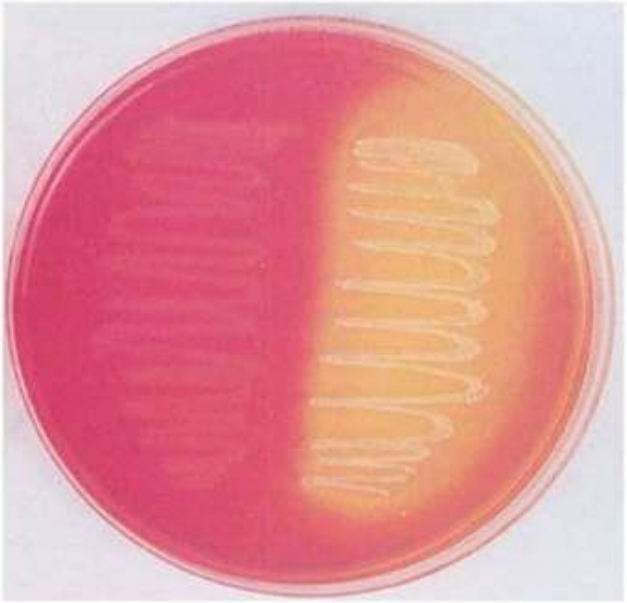
- **Mannitol salt agar (selective differential media for gram positive bacteria :Staphylococcus spp)**
- **Sugar: Mannitol**
- **The inhibitor for gram positive & negative bacteria = Nacl 7.5%**
- **PH-indicator: phenol red**
- **Mannitol fermenter = yellow colony**
- **Mannitol non fermenter = red colony**



***Staphylococcus aureus* on mannitol salt agar**

Mannitol Salt Agar

- Mannitol fermenters includes: Staphylococcus aureus
- Non-mannitol fermenters includes: Staphylococcus epidermidis
- Positive growth but non-mannitol fermenters includes: Micrococcus luteus
- Negative growth includes: Escherichia coli, Pseudomonas aeruginosa



▶ XLD agar (Xylose Lysine Deoxycholate)

Selective differential media for Shigella and Salmonella

- **▶ Sugar : Xylose**
- **▶ PH-indicator: phenol red**
- **▶ H2S indicator : Na - thiosulphate**
- **▶ Gram positive & gram negative inhibitor = Na-Deoxycholate**
- **▶ Shigella non fermenter Xylose = red colony**
- **▶ Salmonella fermenter Xylose = yellow colony with black center**

Xylose lysine Deoxycholate (XLD) Principle...

- *Sodium desoxycholate* inhibits contaminating Gram-positive flora.
- *Xylose* is fermented by practically all coliforms bacteria and *Salmonella*, except for *Shigella* which are thus differentiated from the other species.
- After exhausting xylose, *Salmonella* decarboxylate *lysine* (via lysine decarboxylase) to cadaverine, causing the pH to rise.
- Colonies of *Salmonella* resemble those of *Shigella* in the medium having become basic.
- *Phenol red* is the pH indicator.
- The addition of *lactose* and *sucrose* to the medium enable coliform bacteria to decarboxylate lysine and thereby produce excess acidity, making the indicator turn yellow, favoring their differentiation.
- *Sodium thiosulfate* and *Ferric ammonium citrate* allow the detection of the H_2S producing bacteria.

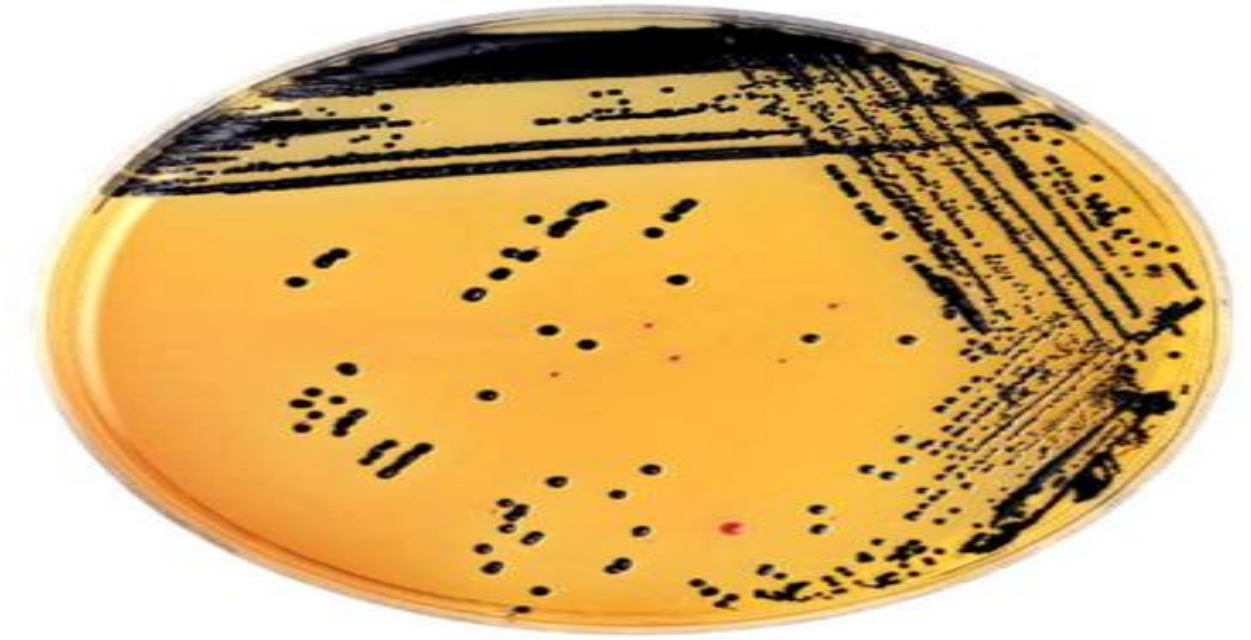
Shigella on XLD.

Salmonella on XLD.

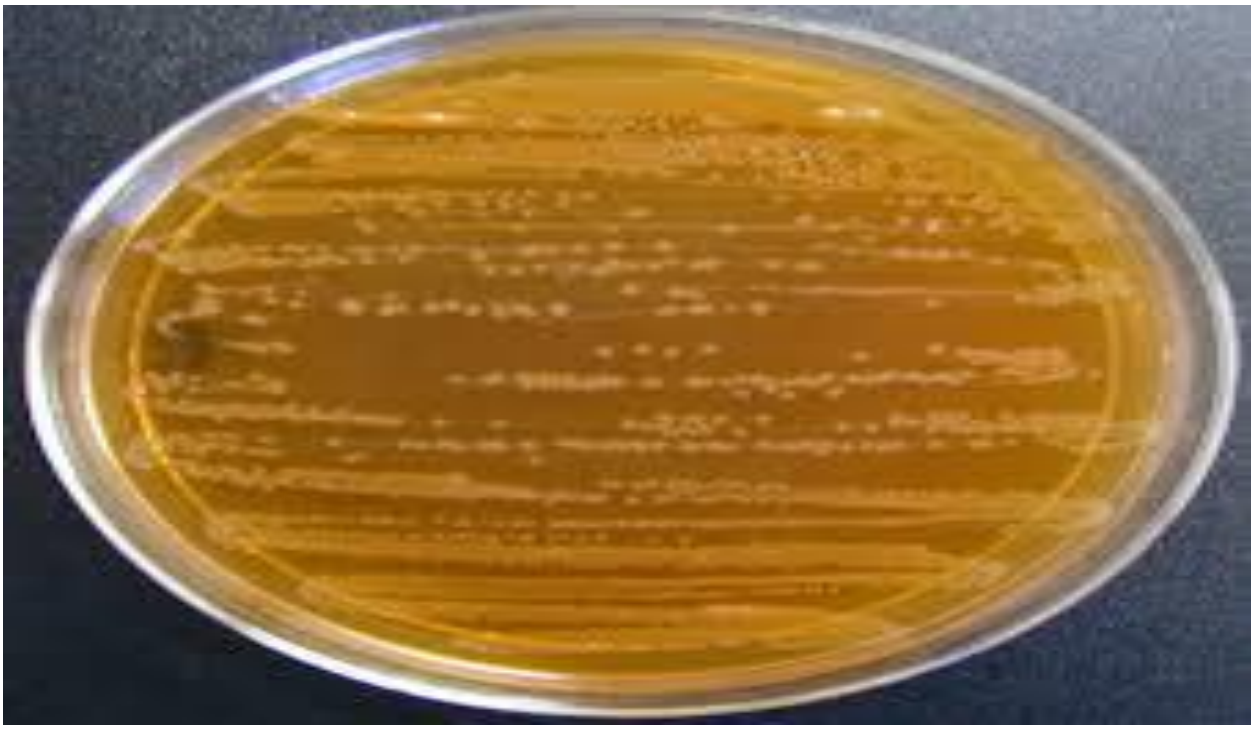
Image Source: Faculty of Health and Medical Sciences - University of Copenhagen, Denmark

▶ S-S agar (Shigella Salmonella agar)

- ▶ **Sugar : lactose**
- ▶ **PH-indicator : Neutral red**
- ▶ **H2S indicator : Na - thiosulphate**
- ▶ **Gram positive & gram negative inhibitor = Brilliant green**
- ▶ **Shigella non fermenter lactose = pale colony**
- ▶ **Salmonella non fermenter lactose = pale colony with black center**

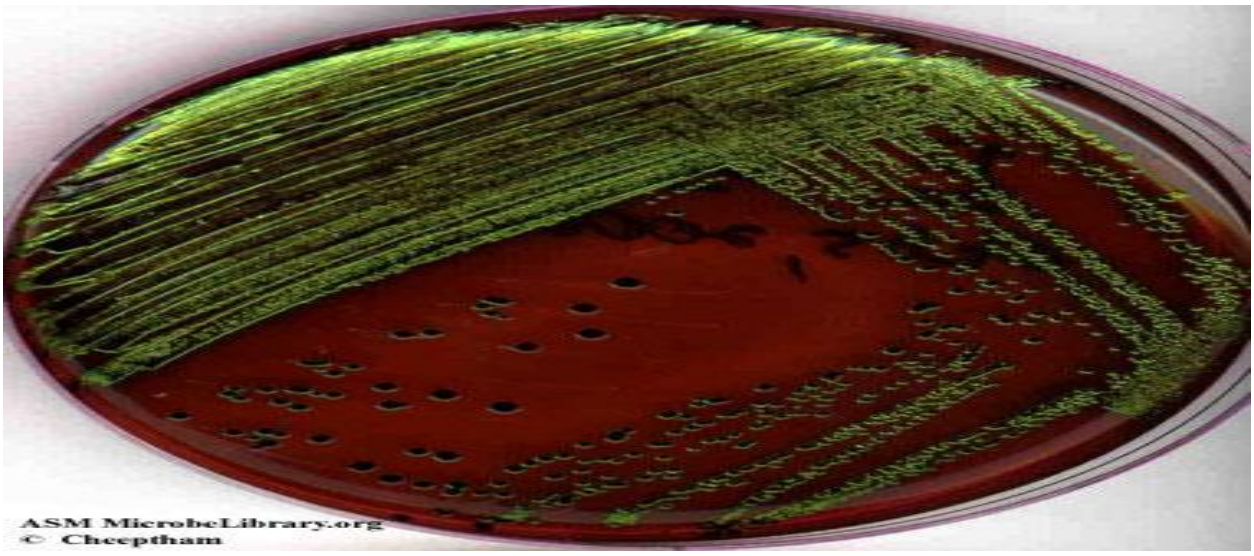


Salmonella on S-S agar pale colonies with black center



Shigella on S-S agar pale colonies

- **Eosin-methylene blue agar: is selective for gram-negative bacteria against gram-positive bacteria .In addition, EMB agar is useful in isolation and differentiation of the various gram-negative bacilli and enteric bacilli, generally known as coliforms and fecal coliforms respectively The bacteria which ferment lactose in the medium form colored colonies, while those that do not ferment lactose appear as colorless colonies EMB agar is used in water quality tests to distinguish coliforms and fecal coliforms that signal possible pathogenic microorganism contamination in water samples .EMB agar is also used to differentiate the organisms in the colon-typhoid-dysentery group:*Escherichia coli* colonies grow with a green metallic sheen with a dark center.**



Escherichia coli on EMB agar (green metallic sheen)

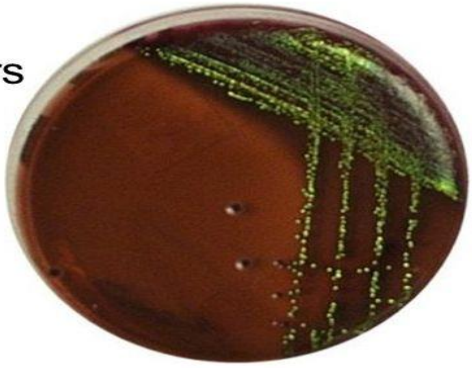
Klebsiella on EMB agar pink – purple mucoid colonies

EMB – Eosin Methylene Blue Agar,
isolate Gram neg. bacteria
Selective – eosin; methylene blue; inhibitory to
Gram + organisms
Differential – lactose fermentation

Metallic green/blue black colonies- (+) vigorous lactose fermentors, acidic

Dark Purple – (+) slower fermentors
acidic

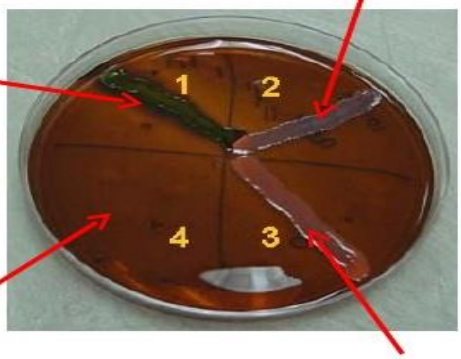
Light pink/colorless –
(-) not fermenting



Levine Eosin-Methylene Blue (**EMB**) Agar

Lactose fermenter
(high acid producer:
black colonies)

Non-lactose fermenter



Gram positive
(growth inhibited)

Lactose fermenter
(low acid producer:
pink colonies)

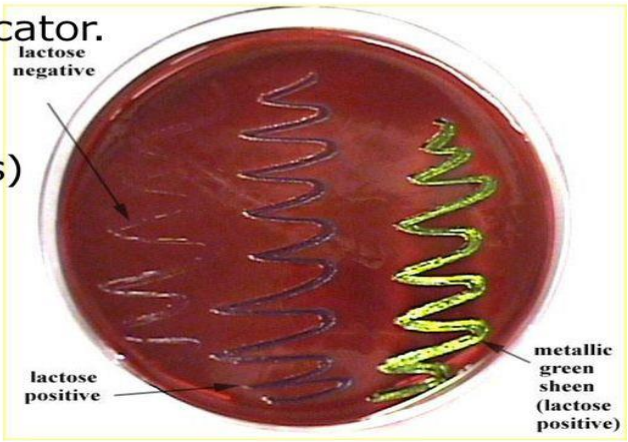
EMB (Eosin Methylene Blue) Agar

Contains peptones, lactose, sucrose, and the **dyes eosin** and **methylene blue**.

Eosin dye inhibits growth of Gram+ bacteria.

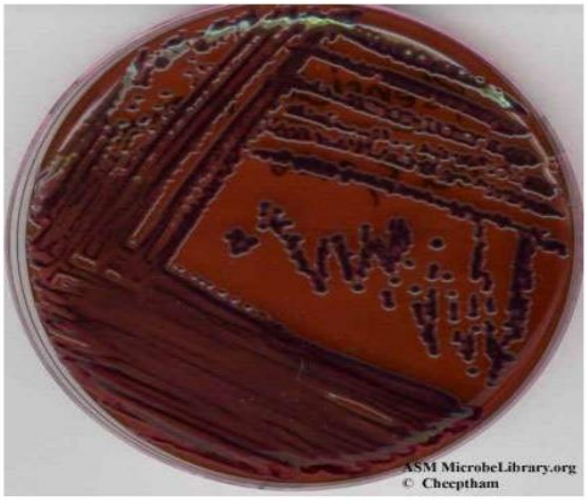
Methylene blue acts as indicator.

Lactose and sucrose are nutrients (fermentable carbohydrates)



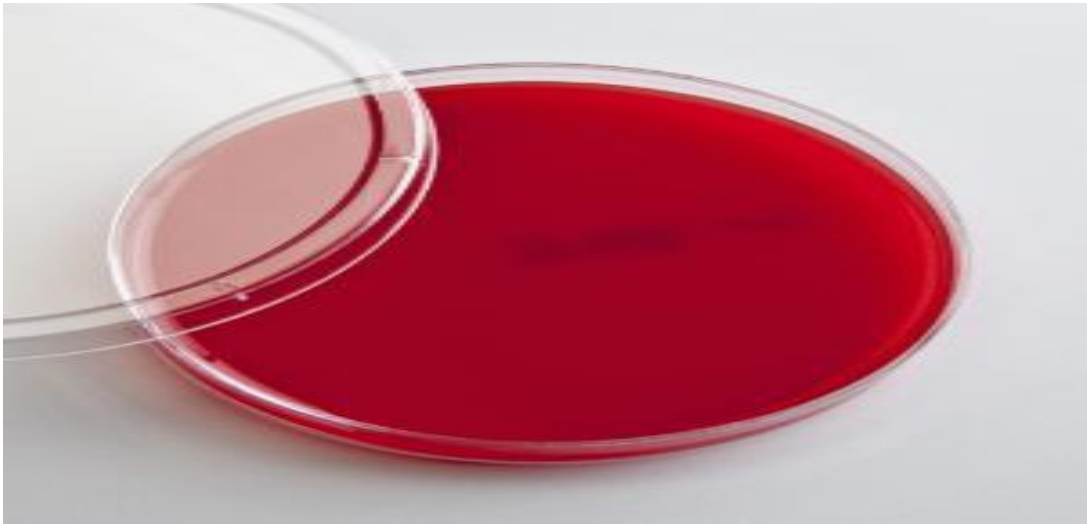
On EMB

- *Klebsiella* species produces large, mucoid, pink to purple colonies with no metallic green sheen on EMB agar.

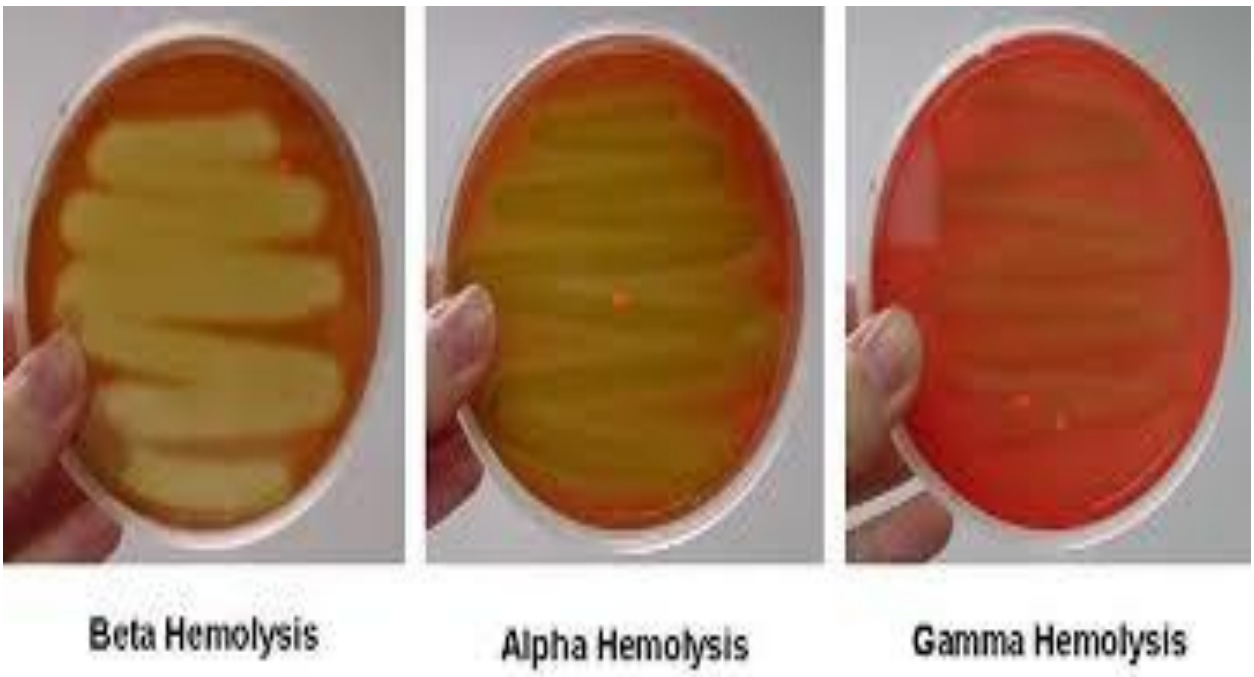


▶ Blood agar (Enrichment differential media)

- ▶ **Enrichment : for fastidious bacteria**
- ▶ **Differential : alpha hemolysis & beta hemolysis**
- ▶ **Add 5 ml blood \100 ml base of blood agar**



Blood agar



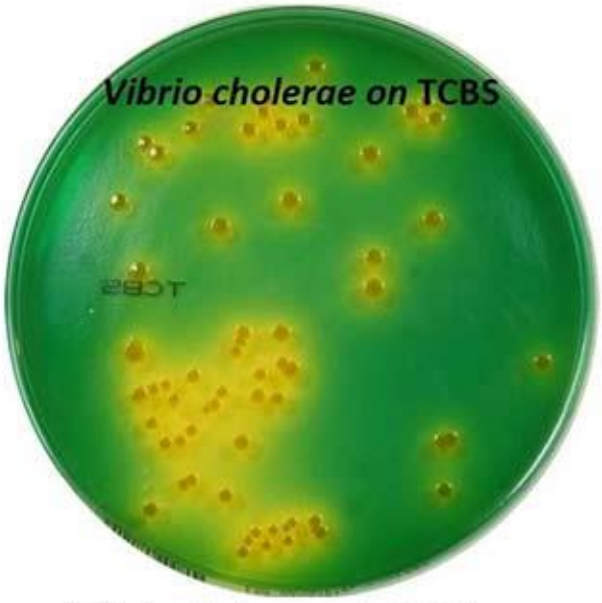
TCBS agar

Thiosulfate–citrate–bile salts–sucrose agar, or TCBS agar, is a type of selective agar culture plate that is used in microbiology laboratories to isolate *Vibrio* species. TCBS agar is highly selective for the isolation of *V. cholerae* and *V. parahaemolyticus* as well as other *Vibrio* species.

TCBS agar contains high concentrations of sodium thiosulfate and sodium citrate to inhibit the growth of *Enterobacteriaceae*. Inhibition of gram-positive bacteria is achieved by the incorporation of ox gall, which is a naturally occurring substance containing a mixture of bile salts and sodium cholate, a pure bile salt. Sodium thiosulfate also serves as a sulfur source and its presence, in combination with ferric citrate, allows for the easy detection of hydrogen sulfide production. Sucrose is included as a fermentable carbohydrate for metabolism by *Vibrio* species. The alkaline pH of the medium enhances the recovery of *V. cholerae* and inhibits the growth of others. Thymol blue and bromothymol blue are included as indicators of pH changes.

Typical colony morphology

- ***V. cholerae*: Large yellow colonies.**
- ***V. parahaemolyticus*: Colonies with blue to green centers.**



Vibrio cholerae on TCBS Agar

Vibrio parahaemolyticus on TCBS Agar