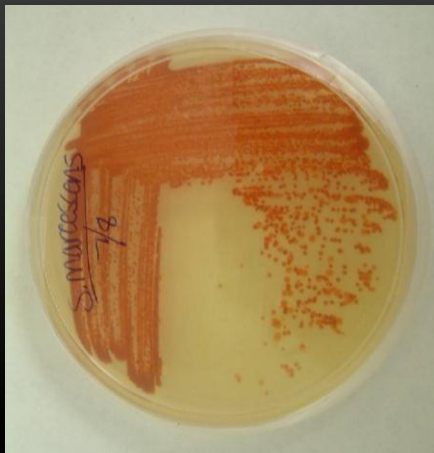
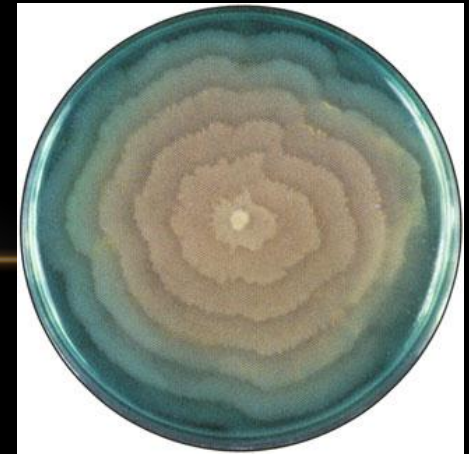




MICROBIOLOGICAL CULTURE & TYPES OF CULTURE MEDIA



Microbiological culture: Method of cultivating microbial organisms by letting them reproduce in predetermined culture media under controlled laboratory conditions.

Culture media: The food material or substances required for growing microorganisms in vitro (outside the body) is called culture medium.

Purpose of culturing

- Isolation of bacteria.
- Properties of bacteria i.e. culturing bacteria is the initial step in studying its morphology and its identification.
- Maintenance of stock cultures.
- Estimate viable counts.

Purpose of culturing

- To test for antibiotic sensitivity.
- To create antigens for laboratory use.
- Certain genetic studies and manipulations of the cells also need that bacteria to be cultured in vitro.
- Culturing on solid media is another convenient way of separating bacteria in mixture.

History of culture media

- Louis Pasteur used simple broths made up of urine or meat extracts.
- Robert Koch realized the importance of solid media and used potato pieces to grow bacteria.
- It was on the suggestion of Fannie Eilshemius, wife of Walther Hesse (who was an assistant to Robert Koch) that agar was used to solidify culture media.

History of culture media

- Before the use of agar, attempts were made to use gelatin as solidifying agent.

Gelatin had some inherent problems....

- ▯ It existed as liquid at normal incubating temperatures (35-37°C)
- ▯ Digested by certain bacteria

Agar

- Frau Hesse
- Used for preparing solid medium
- Obtained from seaweeds.
- No nutritive value
- Not affected by the growth of the bacteria.
- Melts at 98°C & sets at 42°C
- 2% agar is employed in solid medium

TYPES OF CULTURE MEDIA

I. Based on their consistency

- a) solid medium
- b) liquid medium
- c) semi solid medium

II. Based on the constituents/ ingredients

- a) simple medium
- b) complex medium
- c) synthetic or defined medium
- d) Special media

Colony – macroscopically visible collection of millions of bacteria originating from a single bacterial cell.

Special media

- Enriched media
- Enrichment media
- Selective media
- Indicator media
- Differential media
- Sugar media
- Transport media
- Media for biochemical reactions

III. Based on Oxygen requirement

- Aerobic media
- Anaerobic media

Solid media – contains 2% agar

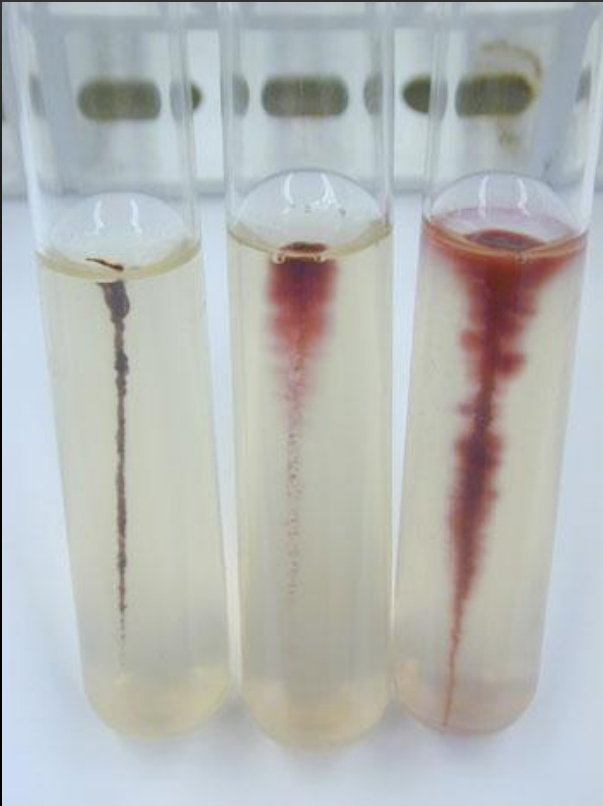
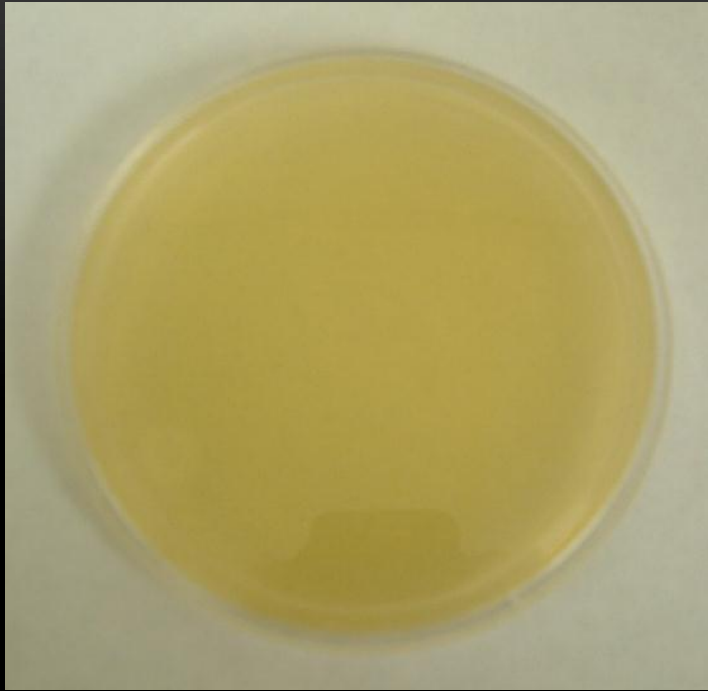
- Colony morphology, pigmentation, hemolysis can be appreciated.
- Eg: Nutrient agar, Blood agar

Liquid media – no agar.

- For inoculum preparation, Blood culture, for the isolation of pathogens from a mixture.
- Eg: Nutrient broth

Semi solid medium (gelatinous) – 0.5% agar.

- Eg: Motility medium



Simple media / basal media

- Eg: NB, NA
- NB consists of peptone, meat extract, NaCl,
- NB + 2% agar = Nutrient agar

Complex media

- Media other than basal media.
- They have added ingredients.
- Provide special nutrient in unknown quantities that are added to bring about a particular characteristic of a microbial strain. Eg: tryptic soy broth, blood agar, and nutrient broth.

Synthetic or defined media

- Media prepared from pure chemical substances and its exact composition is known
- Eg: peptone water – 1% peptone + 0.5% NaCl in water

Enriched media

- Substances like blood, serum, egg are added to the basal medium.
- Used to grow bacteria that are exacting in their nutritional needs.
- Eg: Blood agar, Chocolate agar



Blood agar



Chocolate agar

Enrichment media

- Liquid media used to increase the concentration of certain microbes before culturing them on a solid medium plate.
- Media is incorporated with inhibitory substances to suppress the unwanted organism.
- Eg:
 - **Selenite F Broth** – for the isolation of *Salmonella*, *Shigella*
 - **Alkaline Peptone Water** – for *Vibrio cholerae*



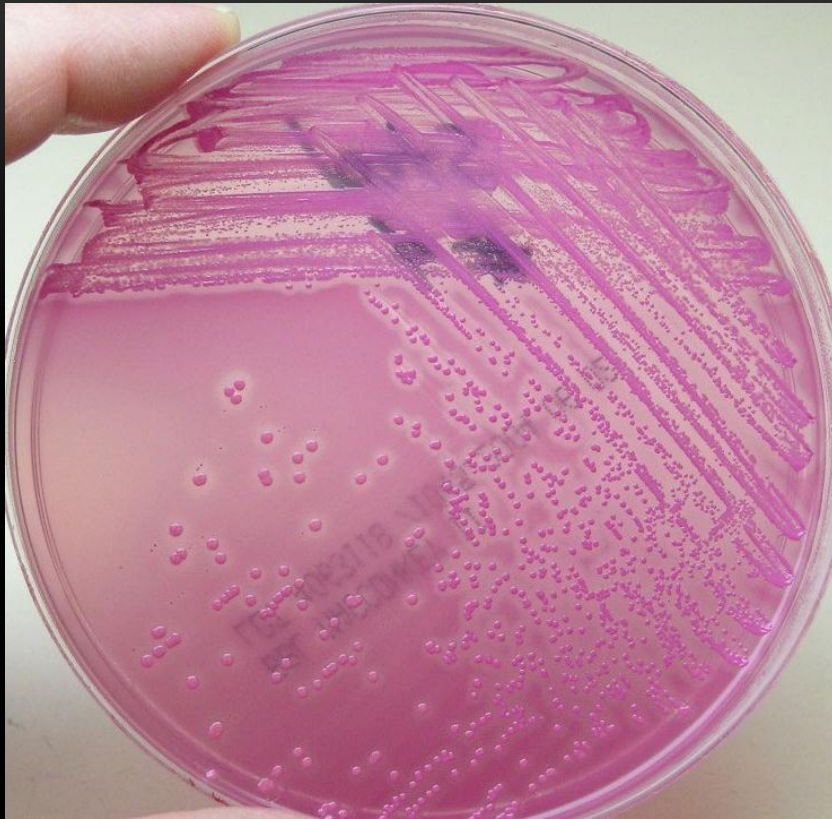
Selective media

- The inhibitory substance is added to a solid media.

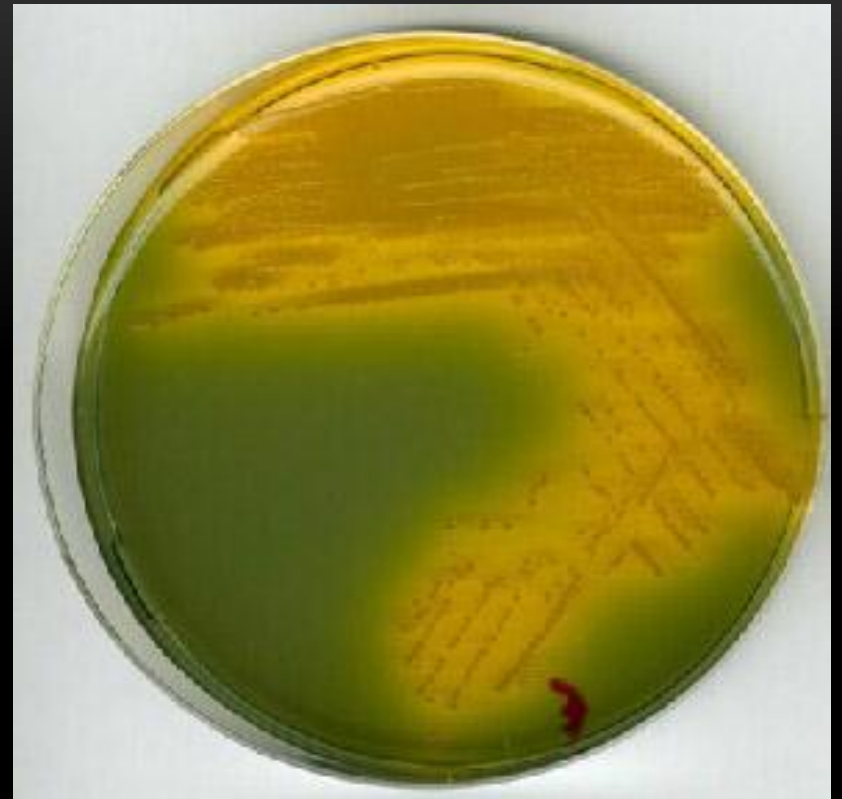
Eg:

- Mac Conkey's medium for gram negative bacteria
- Thiosulfate citrate bile salts sucrose (TCBS) medium – for *V. cholerae*
- Lowenstein Jensen medium – *M. tuberculosis*
- Wilson and Blair medium – *S. typhi*
- Potassium tellurite medium – *Diphtheria bacilli*

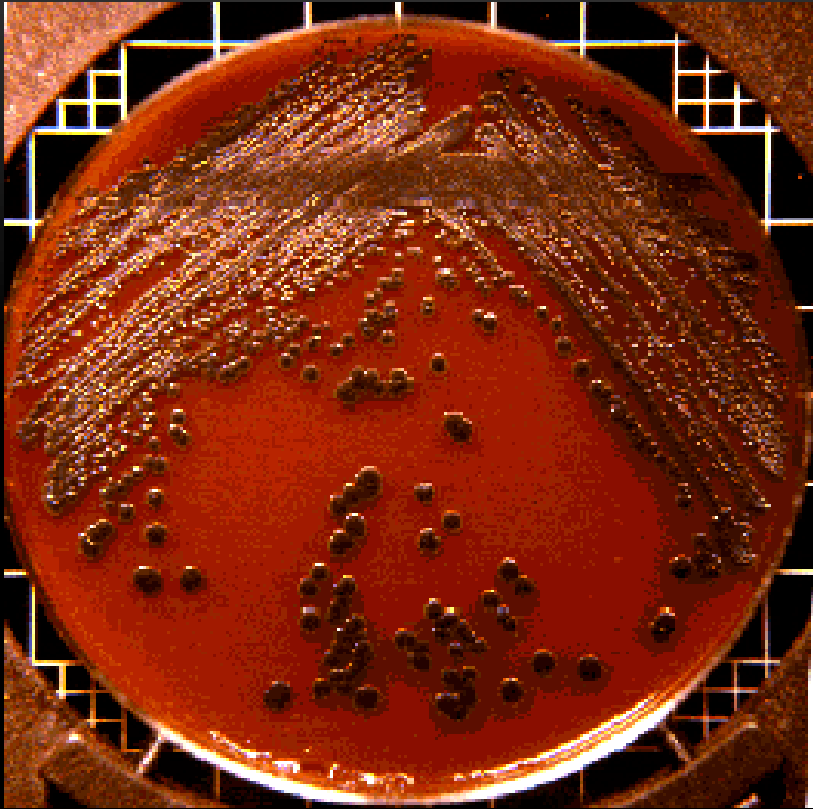
- MacConkey agar:** It differentiates the gram-negative bacteria based on their lactose metabolism. The lactose fermenting bacteria, such as *Escherichia coli*, *Klebsiella spp*, *Citrobacter*, and *Enterobacter* forms pink-red colonies, while lactose non-fermenters, like *Salmonella*, *Shigella*, *Proteus*, *Pseudomonas*, and *Morganella* form colorless colonies.



Mac Conkey's medium



TCBS



Potassium Tellurite media



LJ media

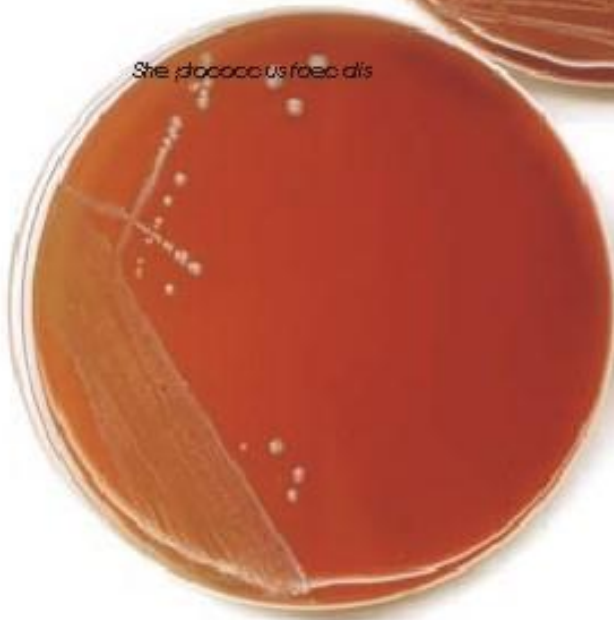
Indicator media

- These media contain an indicator which changes its colour when a bacterium grows in them.
- Eg:
 - Blood agar
 - Mac Conkey's medium
 - Christensen's urease medium

Streptococcus pyogenes



Streptococcus faecalis



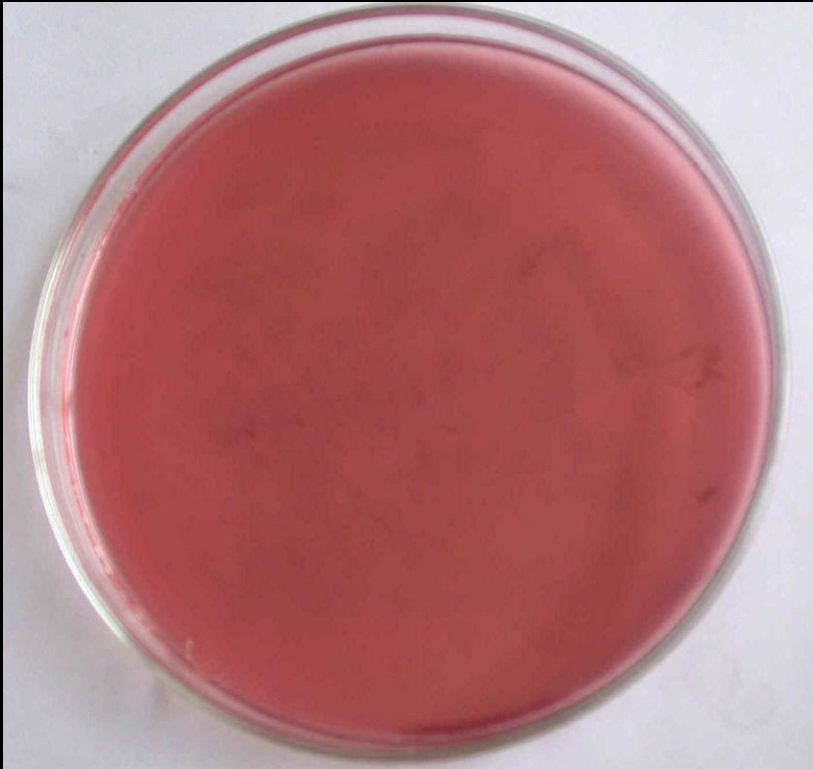
Streptococcus agalactiae





Urease medium

- Lactose fermenters – **Pink** colonies
- Non lactose fermenters – colourless colonies



Transport media

- Media used for transporting the samples.
- Delicate organisms may not survive the time taken for transporting the specimen without a transport media.
- Eg:
 - **Stuart's medium** – non nutrient soft agar gel containing a reducing agent
 - **Buffered glycerol saline** – enteric bacilli



Anaerobic media

- These media are used to grow anaerobic organisms.
- Eg: Robertson's cooked meat medium, Thioglycolate medium.

